(54) **PDE core construct**

PDE Kern Konstrukt

Construction dérivée du noyau de PDE

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Description

[0001]   The invention relates to modified phosphodiesterase (PDE) polypeptides with altered physicochemical properties which are particularly useful in screening assays for the identification of agonists or antagonists of PDEs, the polynucleotides encoding such modified PDE polypeptides, antibodies against such polypeptides, methods of preparation and uses of such polypeptides.

[0002]   The phosphodiesterases (PDEs) represent a family of enzymes that catalyze the hydrolysis of the various cyclic nucleoside monophosphates (including cAMP). These cyclic nucleotides have been found to act as second messengers within the cells by carrying impulses from cell surface receptors having bound various hormones and neurotransmitters to the inside of the cells. The task of phosphodiesterases is to degrade these cyclic mononucleotides once their messenger role is completed thereby regulating the level of cyclic nucleotides within the cells and maintaining cyclic nucleotide homeostasis.

[0003]   Ten families of PDEs have been identified, designated PDE1 to PDE10. Within each family there are two or more related but distinct gene products (A, B, C, etc.) and for each of these alternative mRNA processing gives rise to multiple splice variants, identified by an additional arabic numeral in accordance with the most recent nomenclature recommendation (Molecular Pharmacology 46, 399-405, 1994).

[0004]   All PDE gene products identified so far have one catalytic and a least one regulatory domain. The catalytic domain lies towards the carboxylic acid terminus of each PDE protein and has the greatest homology between the PDE families, being >75% homologous at the amino acid level (Perry and Higgs, Curr. Opinion Chem. Biol. 2, 472-481, 1998). Nevertheless, each of the more than 30 known PDEs have individually distinct substrate specificities, kinetic characteristics, regulatory properties and cellular and subcellular distributions (Houslay and Milligan, Trends in Biochem. Sci. 22, 217-224, 1997).

[0005]   PDEs 4, 7 and 8 are highly specific for cAMP. PDEs 5, 6, 9 and 10 are selective for cGMP. PDE3s bind cAMP and cGMP with similar affinity, but hydrolyse cAMP most efficiently, cGMP rather poorly. PDE3s are, therefore, negatively regulated in their cAMP hydrolysing ability by cGMP. PDEs 1 and 2 hydrolyse both cAMP and cGMP, but with PDE1 the relative efficiencies vary with isoenzyme subtype (Perry and Higgs, Curr. Opinion Chem. Biol. 2,472-481,1998).

[0006]   The amino terminal ends of PDEs consist of the regulatory domains, which are very different both between families and between variants within families. This region contains variously: a binding domain for Ca2+-calmodulin (CaM) in PDE1 ; non-catalytic cGMP-35 binding sites in PDEs 2, 5 and 6; a binding domain for the signalling G-protein transducin in PDE6. The amino terminal region also contains protein- and membrane-targeting sequences in several PDE3s and PDE4s, as well as protein kinase phosphorylation sites in PDEs 1, 3, 4 and 5. These phosphorylation sites are likely to be important in regulation of catalytic activity and/or subcellular location (Perry and Higgs, Curr. Opinion Chem. Biol. 2, 472-481, 1998).

[0007]   Of particular interest to this invention are the PDE4 enzymes. PDE4 enzymes share a common structure, as deduced from their amino acid sequences (Beavo and Reifsnyder, Trends Pharmacol. Sci. 11, 150-155, 1990; Bolger at al., Mol. Cell Biol. 13, 6558-6571, 1993; Houslay, Sullivan and Bolger, Adv. Pharmacol. 44, 225-242, 1998). Members of each gene family (PDE4A, PDE4B, PDE4C, PDE4D) share common C-terminal regions, different for each family, and catalytic domains that for all PDE4 isoforms are very similar (84% homology over about 360 amino acids across all PDE4s; Houslay, Sullivan and Bolger, Adv. Pharmacol. 44, 225-242, 1998).

[0008]   From N-terminus to catalytic region, the sequence in "long form" PDE4s can be divided into 5 regions, three of which are isoform-specific (N-terminal region, linker regions 1 and 2, or LR1 and LR2) and two, more conserved regions, that are broadly similar between all isoforms, the upstream conserved regions 1 and 2 (UCR1 and UCR2). "Short form" PDE4s, e.g. PDE4A1, PDE4B2, PDE4D1, PDE4D2, lack UCR1 and LR1 plus differing amounts of the N-terminal region of UCR2. Throughout all regions are potential phosphorylation sites for a variety of kinases, including PKA (e.g. Ser 54 in human PDE4D3), mitogen activated protein kinases (e.g. Ser 487 of human PDE4B2), casein kinase II (e.g. Ser 489 of PDE4B2) and calcium-diacylglycerol dependent protein kinases (Houslay, Sullivan and Bolger, Adv. Pharmacol. 44, 225-242, 1998). Phosphorylations at some of these sites have been shown to activate the PDEs (e.g. Ser 54), others serve to inhibit. There is also evidence that some phosphorylations serve to prime the enzymes ready for subsequent activation by further phosphorylation at a different site or sites (Houslay, Sullivan and Bolger, Adv. Pharmacol. 44, 225-242, 1998). Other auto-regulatory sites may be found in the N-terminal sequence of certain PDE4s (Bolger at al., Mol. Cell Biol. 13, 6558-6571, 1993, Mc Phee et al., Biochem. J. 310, 965-974,1995).

[0009]   Specific PDE4 inhibition may be useful over a very wide range of disease areas. These include: asthma, atopic dermatitis, depression, reperfusion injury, septic shock, toxic shock, autoimmune diabetes, AIDS, Crohn's disease, multiple sclerosis, cerebral ischemia, psoriasis, allograft rejection, restenosis, stroke, ulcerative colitis, cachexia, cerebral malaria, allergic rhinoconjunctivitis, osteoarthritis, rheutmatoid arthritis, autoimmune encephalomyelitis (Houslay, Sullivan and Bolger, Adv. Pharmacol. 44, 225-242, 1998, WO02/074992). In the area of asthma, PDE4 inhibition helps to increase cAMP in bronchial smooth muscle, thereby producing a modest bronchodilatory effect, of use in the alleviation of asthmatic symptoms. But perhaps most importantly, inhibition of PDE4s is now a recognised method by which to

suppress immune and inflammatory cell responses (Hughes et al. Drug Discov. Today 2, 89-101, 1997; Torphy, Am. J.Respir. Crit. Care Med. 157, 351-370, 1998; Teixeira et al., Trends Pharm. Sci. 18, 164-171,1997).

[0010] PDE4s play major roles in modulating the activity of virtually every cell type involved in the inflammatory process. Immune and inflammatory conditions occur when recruitment of leukocytes from the blood compartment into tissues is either uncontrolled, inappropriate, prolonged or directed against self. In asthma, rheumatoid arthritis and multiple sclerosis, infiltration of tissues with inflammatory cells is prolonged and intense, leading ultimately to severe (and self-perpetuating) damage and loss of function. Acute disregulation of the immune system occurs in such conditions as acute respiratory distress syndrome (ARDS) where an overwhelming and generalised inflammatory response can frequently lead to death. There is also substantial evidence which suggests that inflammation may play a part in defining the extent of injury resulting from reperfusion following ischaemia, at least in brain and lung (Entman and Smith, Cardiovasc. Res. 28, 1301-1311, 1994).

[0011] The PDE4D phosphodiesterases, which are the most preferred focus of the present invention, exist in mammals in the form of isoenzymes (which represent different molecular forms of the same enzyme polypeptide). These phosphodiesterases are localized in the cytosol of the cell and are unassociated with any known membraneous structures. The PDE4D isoenzymes specifically degrade cAMP and are a common target for such pharmacological agents as antidepressants (for example, rolipram). Several splice forms of PDE4D are known. The short forms, PDE4D1 and PDE4D2, lack UCR1 and LR1 sites. Rather than these, interest of the present invention focuses on the long isoforms, of which 6 are known, namely PDE4D3, PDE4D4, PDE4D5, PDE4D6, PDE4D7 and PDE4D8. All of these have in common the LR1 and UCR1 sites and the domains located at the C-terminus of these sites, but they have different N-terminal domains. Isoforms PDE4D6, D7 and D8 were recently disclosed in WO02/074992.

[0012] For identification of compounds that inhibit PDE4D isoenzymes, screening assays are used that are either whole cell assays in which the cells are treated with the inhibitor and cAMP hydrolysis is measured, or in vitro assays which use purified PDE4D isoenzymes either isolated from their natural sources or from cells overexpressing recombinant PDE4D isoenzymes. Such assays are described e.g. in WO00/09504, WO01/35979, WO01/68600 and WO98/45268. WO01/35979 describes the purification of recombinant PDE4D expressed in Saccharomyces Cerevisiae. The use of recombinant native forms of PDE4D for the identification of PDE agonists or antagonists has the disadvantage that the PDE4D poypeptide does not have optimal physicochemical characteristics, especially with respect to aggregate formation. Aggregate formation can lead to protein precipitation and is known to potentially decrease the activity of enzymes, compared to the authentic oligomeric molecules. Thus, aggregation of the native forms of PDE4D polypeptides in screening assays aimed at identifying agonists or antagonists of PDE4D phosphodiesterase activity negatively affects the quality of these assays. In addition, the use of assays based on individual recombinant PDE4D isoforms has the disadvantage of potentially resulting in the identification of agonists or antagonists that are specific for a single isoform of a PDE4D only, instead of agonists or antagonists which act on all long or short form PDE4D isoforms together.

Figure 1: Specific phosphodiesterase activity for the different PDE4D isoforms D3 and D5 to D8, and the PDE4D core construct (DC).

Figure 2: Aggregate formation by different PDE4D isoforms and the core construct. In this figure, the gel filtration profiles of different isoforms of PDE4D (D3, D5, D6, D7, D8), and the core construct (DC) are shown. The first elution peak represents PDE4D aggregates. The pronounced shoulder in the elution profile of DC, where polypeptides of approx. 300,000 Dalton elute, indicates a decrease in aggregation of DC, as compared to the five isoforms of PDE4D.

Figure 3: Quantitation of aggregation of the core construct (DC) of PDE4D, and of the tubulin content of the core construct perparations. In A), the elution profile of DC is shown, with the fractions indicated on the bottom. B) shows an SDS-PAGE of the different fractions from the gelfiltration. C) shows the analysis of optical densities of individual fractions corresponding to the Coomassie stained SDS-PAGE in B).

Figure 4: Differences in aggregation and tubulin content of different isoforms and the core construct of PDE. A) SDS-PAGE gels are shown for DC and five isoforms of PDE4D which were analysed as described above. B) The percentage of non-aggregated PDE4D is shown for the core construct and the isoforms D3 and D5 to D8, based on total PDE4D protein. DC has the highest percentage of non-aggregated PDE4D, as compared to the five isoforms. C) This figure shows the ratio between PDE4D and tubulin for DC and the isoforms D3 and D5 to D8, based on total PDE mass and total tubulin mass. As can be seen, the tubulin content of DC is significantly lower than that of the five PDE4D isoforms indicated.

Figure 5: Activity assay. Phosphodiesterase activity is shown both for the PDE4D core construct (DC) and PDE4D3 (D3).

Figure 6: Rolipram inhibition. Phosphodiesterase activity of the PDE4D core construct (DC) can be inhibited by Rolipram. The IC50 of DC phosphodiesterase activity by Rolipram inhibition was 0.34+/- 0.06 mM.

[0013] The term "polypeptide" as used herein, refers to a polymer of amino acids, and not to a specific length. Thus, peptides, oligopeptides and proteins are included within the definition of polypeptide.

[0014] The term "physicochemical properties" as used herein refers to the propensity of polypeptides to monomer formation, oligomer formation or aggregate formation.

[0015] The terms "aggregation" and "aggregate formation" as used herein are used interchangeably and refer to the type of association of polypeptide chains which, in contrast to oligomer formation, is an irreversible process and does not follow the law of mass interaction. Aggregation progresses in a time dependent manner and does not stop when a defined number of chains have associated. Aggregation of polypeptides can lead to protein precipitation because the molecular size of the aggregate is so dramatically increased that the solubility of the molecules is impaired. Aggregation is also known to potentially decrease the activity of the enzyme as compared to authentic oligomeric molecules.

[0016] The term "oligomer formation" as used herein refers to a reversible association of polypeptide chains whereby the ratio of oligomeric proteins and monomeric proteins at a certain protein concentration and at thermodynamic equilibrium is described by the association constant.

[0017] The term "non-aggregated" refers to polypeptides that are present either as oligomers or monomers or a mixture of oligomers and monomers at equilibrium according to the law of mass interaction.

[0018] The term "improved physicochemical properties" as used herein refers to a decrease in aggregate formation of polypeptides. For the purpose of definition, aggregate formation of the polypeptides of this invention is determined by quantitation of the fractions of a gelfiltration experiment in which aggregated PDE elutes, and of the fractions in which non-aggregated PDE elutes. The degree of aggregation is calculated in percent of non-aggregated PDE based on total (aggregated and non-aggregated) PDE. For the purpose of the definition of aggregation, aggregated PDE is considered to elute in fractions 2 to 4, in which aggregated molecules of at least 2 Mio Daltons elute, and non-aggregated PDE is considered to elute in fractions 5 to 11, corresponding to the fractions in which molecules of about 0.3 Mio Daltons elute, when the following conditions are used for gel filtration: 50 μl of Ni-NTA agarose purified isoform preparation are injected into a Superose 12 size exclusion column (type PC3.2/30; Amersham Pharmacia Biotech), equilibrated with 50 mM TrisHCl pH 7.7, 100 mM NaCl, 0.5 mM MgCl2 at a flow rate of 0.1 ml/min at 4 °C. Chromatograms are recorded at 278 nm. Starting from the elution volume onwards, the column eluate is collected as 50 μl fractions.

[0019] The term "long form PDE" as used herein refers to PDE isoforms which comprise an isoform specific N-terminal domain, linker regions LR1 and/or LR2, and upstream conserved regions UCR1 and UCR2.

[0020] The term "UCR1 start" as used herein refers to the first amino acid of the UCR1 domain of PDE4D, as annotated in SEQ ID No. 1 to 7.

[0021] The term "LF1 site" as used herein refers to the first amino acid of the splice site of PDE4D as indicated in SEQ ID No. 1-7.

[0022] The term "amino acids upstream of LF1" refers to the position of an amino acid counted from the LF-1 site towards the amino terminus of the native protein.

[0023] The term "D3" as used herein refers to the PDE4D3 isoform. The term "D4" as used herein refers to the PDE4D4 isoform. The term "D5" as used herein refers to the PDE4D5 isoform. The term "D6" as used herein refers to the PDE4D6 isoform as provided by WO02/074992. The term "D7" as used herein refers to the PDE4D7 isoform as provided by WO02/074992. The term "D8" as used herein refers to the PDE4D8 isoform as provided by WO02/074992. The polypeptide sequences of the human PDE4D isoforms D3, D4, D5, D6, D7, and D8 are shown in SEQ ID No.1 and 3-7.

[0024] The term "Ser54" as used herein refers to the Ser residue in position 54 of PDE4D3, and to the Ser residue in the respective position of any of the other PDE polypeptides. The position corresponding to Ser54 of PDE4D3 is annotated in SEQ ID No. 1 to 7 for the different isoforms and the core sequence which is common to all the PDE4D isoforms.

[0025] The term "Ser579" as used herein refers to the Ser residue in position 579 of PDE4D3, and to the Ser residue in the respective position of any of the other PDE polypeptides. The position corresponding to Ser579 of PDE4D3 is annotated in SEQ ID No. 1 to 7 for the different isoforms and the core sequence which is common to all the PDE4D isoforms.

[0026] The term "tubulin association" as used herein refers to the tubulin content of purified PDE preparations.

[0027] As used herein, the term "agonist" also refers to activators, and the term "antagonist" also refers to inhibitors of PDE phosphodiesterase activity.

[0028] The disadvantage of native recombinant PDE polypeptides resides in the formation of aggregates of the polypeptides, which gives rise to sub-optimal conditions for the identification of modulators of PDE phosphodiesterase activity in screening assays since aggregate formation is known to lead to polypeptide precipitation and to potentially impair activity of a polypeptide. The present invention is directed to the generation of long form PDE[4] polypeptides with improved physicochemical properties which are particularly useful for the purpose of identifying agonists or antagonists of these enzymes.

[0029] The problem of aggregate formation of recombinant long form PDE4 polypeptides was overcome in the present invention by generating a polypeptide comprising a long form PDE4 polypeptide sequence with the exception of the PDE4A polypeptide sequence, with an amino-terminal deletion between the L71 splice site and the UCR1 start said polypeptide exhibiting decreased aggregate formation, as shown in Figures 2 and 3. These polypeptides have a specific activity that is comparable to the specific activity of the native recombinant proteins, as is shown in Figure 1. In a preferred embodiment, the invention provides a polypeptide comprising a long form PDE4 polypeptide sequence, with an amino-terminal deletion wherein the proportion of non-aggregated long form PDE4 in the total protein preparation is from 55 %, preferably from 60%, more preferably from 65%, most perferably from 68% to 100%, preferably to 90 %, more preferably to 80 %, most preferably to 70% of total protein, as determined by the quantitation of eluted fractions from a gel filtration of the long form PDE4 fractions. Quantitation is performed as follows: Equal volume aliquots of fractions from Size Exclusion Chromatography, run as hereinbefore described, are analyzed by SDS-PAGE (one gel per run and isoform, Figure 3). Optical densities of Coomassie stained long form PDE4 are integrated as follows: After electrophoresis the Coomassie stained polyacrylamide gel was imaged by a video imaging system (Figure 1). Optical densities of PDE bands were integrated using a Macintosh computer and the public domain software "NIH Image", version 1.61 (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/). The integrated arbitrary units per PDE band as returned by the software reflect the relative PDE concentrations within the original pools. Integrated densities from PDE bands of fractions 2-4 are added up, giving a relative amount of aggregated PDE, PDE_{agg} (Figure 3). These fractions contain molecules of at least 2 Mio Daltons. Similarly, integrated densities from PDE bands of fractions 5-11 are added up, giving a relative amount of non-aggregated PDE, $PDE_{non-agg}$. In fractions 5-11, molecules in the range of 0.3 Mio Daltons elute. Relative amount of total PDE, PDE_{total}, is given by the sum of PDE_{agg} and $PDE_{non-agg}$. The percentage of non-aggregated PDE is given by the ratio of $PDE_{non-agg}$ and PDE_{total}, multiplied by 100.

[0030] In an even more preferred embodiment, the polypeptide sequence is a PDE4D polypeptide sequence, preferably a long form PDE4D polypeptide sequence.

[0031] Methods of generating amino-terminal deletion mutants of polypeptides are well known. Such deletion mutants can be generated by using site directed mutagenesis, e.g. by using PCR (Sambrook et al., Molecular Cloning: A Laboratory Manual (1989), Cold Spring Harbor Laboratory Press, New York, USA).

[0032] In one embodiment of the present invention, the polypeptide comprises a PDE polypeptide sequence starting between 25 amino acids, preferably between 20 amino acids, more preferably between 15 amino acids, even more perferably between 10 amino acids, most preferably between 5 amino acids upstream of LF1 and the LF1 site.

[0033] In another embodiment the PDE long form polypeptide sequence starts at any amino acid located between the LF1 splice site and the first amino acid of the UCR1 start of the native long form PDE4 polypeptide. In the case of the most preferred embodiment of the long form PDE4 polypeptide, PDE4D, these sites are identical in all of the long form PDE4D isoforms.

[0034] In another more preferred embodiment of the present invention, the amino-terminal deletion is such that the PDE polypeptide sequence starts at the LF1 splice site of the native PDE polypeptide. This PDE polypeptide sequence is referred to as the "core construct". The core construct spans a sequence of PDE which is shared by all isoforms. The core construct sequence of PDE4D is shown in SEQ ID No. 2.

[0035] In another more preferred embodiment of the present invention, the start of the PDE polypeptide sequence is located 13 amino acids upstream of the UCR1 start of the native PDE polypeptide. The location of this position in the long form PDE4D isoforms and the core construct of PDE4D is shown in SEQ ID No. 1-7.

[0036] The basis for the generation of the above mentioned long form PDE4 polypeptide with an amino-terminal deletion and improved physicochemical characteristics can be any isoform of long form PDE4, with the exception of PDE4A. For the most perferred embodiment the long form PDE4 polypeptide sequence is an isoform of PDE4D selected from the group consisting of D3, D4, D5, D6, D7, and D8. However, the present invention also extends to further isoforms of long form PDE4D that have not yet been identified.

[0037] Also of use to the present invention are mutants of the long form PDE4 polypeptide herein before described. Of particular interest is a long form PDE[4] polypeptide sequence comprising one or more mutations of Serine residues. Preferably, said Serines are mutated to either Alanine or Aspartic acid. In a most preferred embodiment, said Serine residues are selected from the group consisting of Ser54 and Ser579. Such Serines represent targets for phosphorylation of long form PDE[4] polypeptides. Phosphorylation of some of these sites has been shown to activate or ihibit the phosphodiesterase activity of PDEs.

[0038] Methods of generating amino acid substitutions in polypeptides are well known to those skilled in the art of Molecular Biology, e.g. methods such as site directed mutagenesis as described in Sambrook et al., Molecular Cloning: A Laboratory Manual (1989), Cold Spring Harbor Laboratory Press, New York, USA.

[0039] In another preferred embodiment of the present invention, the long form PDE[4] polypeptide hereinbefore described exhibits decreased tubulin association, thus allowing to obtain purer long form PDE[4] polypeptide preparations. To determine the decrease in tubulin association, the ratio of PDE molecules per tubulin molecules in the preparation of PDE polypeptide is calculated by quantitation of SDS-PAGE. Preferably, the ratio of PDE molecules per tubulin

molecules is at least 5, preferably at least 10, more preferably at least 15, even more preferably at least 20, most preferably at least 25 molecules of PDE per molecules of tubulin. The tubulin content of PDE preparations is determined as follows: Equal volume aliquots of fractions from SEC are analyzed by SDS-PAGE (one gel per run and isoform, Figure 3). Optical densities of Coomassie stained PDE and tubulin bands are integrated as described hereinbefore for PDE aggregation quantitation. Integrated densities from PDE bands of fractions 2-11 are added up, giving a relative amount of total PDE, PDE_{total}. Likewise, integrated densities from tubulin bands of fractions 2-11 are added up, giving $tubulin_{2-11}$, and thereby the relative amount of total tubulin, $tubulin_{total}$. The ratio of PDE and tubulin (Figure 4C) is identical with the ratio of the relative amount of total PDE4D, PDE_{tot}, and relative amount of total tubulin, $tubulin_{total}$, on a mass per mass basis.

[0040] The long form PDE^4 polypeptides from which the peptide of this invention is generated by amino-terminal deletion as hereinbefore described include polypeptides that are substantially homologous or identical to the polypeptides shown in SEQ ID No. 1-7 and have, in their native form, the phosphodiesterase activity and specificity long form of PDE^4. In particular, these long form PDE^4 polypeptides also include the previously published polypeptide sequences of PDE4D3 to D8 as defined in U50159 (D3), L20969 (D4), S:1059276 (D5) and the sequences for D6, D7 and D8 as disclosed in WO02/074992. As used herein, two polypeptides (or a region of the polypeptides) are substantially homologous or identical when the amino acid sequences are at least 45-55%, typically at least 70-75%, more typically at least 80-85%, and most typically greater than 90% or more homologous or identical. To determine the percent homology or identity of two amino acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in the sequence of one polypeptide for optimal alignment with the other polypeptide or nucleic acid molecule). The amino acid residues or nucleotides at corresponding amino acid positions are then compared. When a position in one sequence is occupied by the same amino acid residue as the corresponding position in the other sequence, then the molecules are homologous at that position. As used herein, amino acid "homology" is equivalent to amino acid "identity". The percent homology between the two sequences is a function of the number of identical positions shared by the sequences (i.e., percent homology equals the number of identical positions/total number of positions times 100). The long form PDE^4 polypeptide sequences of the present invention are preferably mammalian, most preferably human. The present invention also includes variants of the polypeptides hereinbefore described with deletions, insertions, inversions, repeats, and substitutions selected according to general rules known in the art so as to have little effect on activity. For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie, Science 247 (1990), 1306-1310, wherein the authors indicate that there are two main strategies for studying the tolerance of an amino acid sequence to change.

[0041] The present invention provides polynucleotides that comprise polynucleotide sequences that encode the long form PDE^4 polypeptide sequences hereinbefore described. As an example for a sequence comprised in such a polynucleotide, the polynucleotide sequence encoding the "core construct" of PDE4D is shown in SEQ ID No. 8. However, the present invention relates to all polynucleotides comprising polynucleotide sequences encoding any of the PDE polypeptide sequences hereinbefore described.

[0042] The present invention provides an expression vector or virus comprising a polynucleotide sequence that encodes the long form PDE^4 polypeptide sequences hereinbefore described and which is capable of directing expression of the polynucleotide sequence in a compatible prokaryotic or eukaryotic host cell. The invention also provides a prokaryotic or eukaryotic host cell transformed or infected with such an expression vector or virus. The invention further provides a process for the production of such a polypeptide comprising culturing the host cell expressing the long form PDE^4 polypeptide sequences hereinbefore described in a suitable medium so that said polypeptide is expressed, and purifying said polypeptide from the cells. In a preferred embodiment, the virus used for the expression of a modified long form PDE^4 polypeptide is a recombinant baculovirus. Methods of generating recombinant baculoviruses for expression of recombinant proteins are well known to the man skilled in the art. Preferably, the recombinant baculovirus is based on pFASTBac as the transfer vector. The host cell of a preferred embodiment of the invention is an insect cell. Insect cells that can be infected with recombinant baculoviruses for the purpose of expressing recombinant proteins are well known in the art. The preferred process for the production of a modified long form PDE^4 polypeptide is a process comprising culturing the insect cell expressing the long form PDE^4 polypeptide sequences hereinbefore described in a suitable medium so that said polypeptide is expressed, and purifying said polypeptide from the insect cell. To facilitate purification, the long form PDE4 polypeptide may also comprise a tag. Different types of such tags and the methods to join them with polynucleotide sequences encoding a polypeptide are well known to the person skilled in the art, e.g. myc-tag, FLAG-tag, six Histidine tags (hereinafter referred to as 6xHis tag). A review on tagging of proteins can be found e.g. in Fritze and Anderson, Methods in Enzymology (2000) 327, 3-16. It is well known that such tags may be joined to either side of the polypeptide. Some tags are known which may also be removed enzymatically from the purified polypeptide, e.g. FLAG-tag fused to the amino-terminus of a protein may be removed by digestion with bovine enterokinase (Hopp, T.P.(1988) Biotechnology, 6, 1204-1210). In a preferred embodiment the long from PDE4 polypeptides hereinbefore described are linked at their C-terminus to a 6xHis tag. The use of such 6xHis tags for ease of purification of recombinant polypeptides, and methods of purification of 6xHis tagged polypeptides are well known (e.g. in Sisk et al. (1994) J. Virol.

68, 766, and Petty, K.J. (1996) in: Ausubel, F.M. et al., eds. Current Protocols in Molecular Biology, Vol. 2, New York: John Wiley and Sons).

[0043] In a preferred embodiment, the polypeptide of the present invention is purified. As used herein, a polypeptide is said to be "purified" when it is substantially free of cellular material when it it isolated from recombinant and non-recombinant cells. A polypeptide, however, can be joined to another polypeptide with which it is not normally associated in a cell (e.g. in a "fusion protein") and still be "purified". Preferably, the polypeptides of the present invention are purified. The polypeptides of the invention can be purified to homogeneity. It is understood, however, that preparations in which the polypeptide is not purified to homogeneity are useful. The critical feature is that the preparation allows for the desired function of the polypeptide, even in the presence of considerable amounts of other components. Thus, the invention encompasses various degrees of purity. In one embodiment, the language "substantially free of cellular material" includes preparations of the polypeptide having less than about 30% (by dry weight) other proteins (i.e., contaminating protein), less than about 20% other proteins, less than about 10% other proteins, or less than about 5% other proteins. When a polypeptide is recombinantly produced, it can also be substantially free of culture medium, i.e., culture medium represents less than about 20%, less than about 10%, or less than about 5% of the volume of the polypeptide preparation.

[0044] The present invention also provides for a use of the polypeptides hereinbefore described for identifying an agonist or antagonist of phosphodiesterase activity.

[0045] The polypeptides of the present invention can be used for a process of identifying and obtaining a drug candidate for therapy of a vascular disorder, said process comprising measuring the activation or inhibition of the phosphodiesterase activity of any of the above mentioned polypeptides. Different methods for measuring phosphodiesterase activity are known, eg. from Owens et al., Biochem. J. (1997) 326, 53-60. Such a drug candidate may be a compound that is either an agonist or an antagonist of the phosphodiesterase activity of PDE.

[0046] The present invention also provides a screening assay for the identification of agonists or antagonists of phosphodiesterase activity comprising at least one of the above described polypeptides. In addition, the polypeptides hereinbefore described can be used for crystallization. Crystals of the polypeptides are useful for assaying the interaction of compounds with phosphodiesterases.

[0047] Furthermore, the present invention provides a kit for identifying agonists or antagonists of phosphodiesterase activity comprising at least one of the polypeptides hereinbefore described, and a radioactive or IMKP - FP phosphodiesterase kit.

[0048] The invention will be further described by the following non-limiting examples.

Examples

[0049] In the following the term "isoform" relates to the natural PDE4D splice variants D3 and D5-D8 and the invented core variant, DC.

Cloning of the PDE4D isoforms 3-8 and the core construct

Core construct:

[0050] A cDNA encoding the core fragment that is common for all the PDE4D isoforms 3-8 starting with the amino acid sequence FDV carboxyterminal to the LF1 splice site was generated by PCR using a 5' oligonucleotide with a HindIII cloning site (gatgaattcaagcttttttgatgtggacaatggcaca) introducing two additional amino acids (K and L) in front of the FDV sequence. At the 3' end a set of primers was used that either generated the native sequence (gtgatatctcattat-cacgtgtcaggagaacgatcatctatgaca) or added a sequence encoding 6xHis residues (gtgatatctcattatcaa tgggatggtgatggt-gcgtgtcaggagaacgatcatctatgac) to enable rapid purification of the recombinant proteins. The cDNA encoding the core construct was cloned as a EcoRI-EcoRV fragment into the expression vector pENTR™1a (GIBCO/BRL)
Isoforms (except core construct):

The DNA fragments encoding the isoform specific N-termini were generated by using synthetic oligonucleotides with terminal restriction enzyme sites for EcoRI and HindIII incorporated for directional cloning. These isoform specific DNA fragments were fused to the core construct sequence via the HindIII site introducing two additional amino acid residues (K and L). The integrity of the clones was confirmed by DNA sequencing prior to expression.

Expression of the PDE4D isoforms and the core construct in insect cells

[0051] The cDNAs were cloned into the pFASTBAC1 vector (Life Technologies. Inc) for expression in insect cells and the products were confirmed by sequencing. After recombination into the baculovirus genome the purified viral DNAs were transformed into the insect cells. Sf9 cells were cultured at 27°C in TC100 medium (BioWhittaker) with 5% (v/v)

fetal calf serum. Virus stocks were generated with a titer of 1.5×10^9 pfu/ml. For large scale production of the isoforms 1-24 L fermentations of Sf9 cells were infected with a MOI of 1.

[0052] In one example, the 6xHis tagged PDE4D polypeptides DC, D3, D5, D6, D7 and D8 were produced in Sf9 cells in 1L spinner flasks using SF1 medium in the absence of serum. Infected cells were harvested 3 days after infection with the recombinant baculoviruses.

[0053] In another example, the PDE4D core construct DC was produced in a 24 L Airlifter Fermenter with 15 L medium (SF1 with 0% serum), 0.15 L lipids and 9 L Sf9 cells. During the entire fermentation procedure the cells were cultured at pH 6.2, $27.0 +/- 0.2 °C$ and a pO_2 of $30.0 +/- 0.5 \%$. Cells were grown for 3 days. The cell number at infection was 2.3×10^6 cells/ml. Cells were infected with 450 ml recombinant baculovirus. Cells were harvested at 68h post infection and the cell pellet as well as the concentrated supernatant stored at $-80 °C$ until further processing.

Purification of 6xHis tagged PDE4D isoforms

[0054] Sf9 cells from 1 liter culture broth, overexpressing the respective isoform, were resuspended on ice in 50 ml 50 mM HEPES pH 7.8, 300 mM NaCl 10 mM imidazole, 1 mM DTT, supplemented with protease inhibitors (one protease inhibitor cocktail tablet "complete, EDTA-free"; Roche). Opening of cells was performed by use of a 50 ml Dounce homogenizator and the homogeneous mixture was centrifuged for 1 hour at 70'000 g and $4 °C$ (Kontron TFT 45.94 rotor at 30'000 rpm). The supernate was filtrated through a filter with a pore size of 1.2 μM (Minisart; Sartorius, Germany) and then applied to a 6 ml Ni-NTA agarose column at 2 ml/min. After equilibration with 50 mM HEPES pH 7.8, 300 mM NaCl, 10 mM imidazole, protein was eluted with a linear 30 ml gradient from 10 to 230 mM imidazole in the same buffer. Fractions containing the PDE4D isoform as analyzed by Coomassie stained SDS-PAGE were pooled and stored frozen at $-80 °C$. Fresh Ni-NTA agarose material was used for every different PDE4D isoform preparation in order to prevent cross contamination of isoforms.

Specific activities of 6xHis tagged PDE4D isoforms

[0055] Relative concentrations of Ni-NTA agarose purified isoform preparations were estimated by SDS-PAGE. Equal volume amounts of isoform preparations were applied to a gradient gel (4-12% NuPage; Invitrogen). After electrophoresis the Coomassie stained polyacrylamide gel was imaged by a video imaging system (Figure 1). Optical densities of PDE4D bands were integrated using a Macintosh computer and the public domain software "NIH Image", version 1.61 (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/). The integrated arbitrary units per PDE4D band as returned by the software reflect the relative PDE4D concentrations within the original pools (Figure 1, "amount"). Identities of PDE4D and tubulin bands had been verified by independent SDS-PAGE, excision of corresponding bands, trypsin cleavage and identification of tryptic peptides by MALDI-MS.

[0056] Activities of equal volume amounts of 10^6-fold diluted purified isoforms were determined by use of a commercial radioactive phosphodiesterase assay (cAMP-dependent phosphodiesterase [3H] assay; Amersham Pharmacia Biotech), following the instructions of the manufacturer. The obtained arbitrary activity units reflect the relative PDE4D activities within the original pools (Figure 1, "activity").

[0057] Relative specific activities of PDE4D isoforms were calculated by dividing relative activity values by relative concentration values (Figure 1, "specific activity").

Qualitative investigation of aggregation by size exclusion chromatography (SEC)

[0058] 50 μl of Ni-NTA agarose purified isoform preparation was injected into a Superose 12 size exclusion column (type PC3.2/30; Amersham Pharmacia Biotech), equilibrated with 50 mM TrisHCl pH 7.7, 100 mM NaCl, 0.5 mM MgCl2 at a flow rate of 0.1 ml/min at $4 °C$. Chromatograms were recorded at 278 nm. Starting from the elution volume, the column eluate was collected as 50 μl fractions (Figure 2).

Quantification of aggregates and tubulin content

[0059] Equal volume aliquots of fractions from SEC were analyzed by SDS-PAGE (one gel per run and isoform, Figure 3). Optical densities of Coomassie stained PDE4D and tubulin bands were integrated as described above. Integrated densities from PDE4D bands of fractions 2-4 were added up, giving a relative amount of aggregated PDE4D, PDE_{agg} (Figure 3). Similarly, integrated densities from PDE4D bands of fractions 5-11 were added up, giving a relative amount of non-aggregated PDE4D, $PDE_{non-agg}$. Relative amount of total PDE4D, PDE_{total}, is given by the sum of PDE_{agg} and $PDE_{non-agg}$. The percentage of non-aggregated PDE is given by the ratio of $PDE_{non-agg}$ and PDE_{total}, multiplied by 100 (Figure 4B).

[0060] Similarly, integrated densities from tubulin bands of fractions 2-11 were added up, giving $tubulin_{2-11}$, and thereby

the relative amount of total tubulin, tubulin$_{total}$. The ratio of PDE and tubulin (Figure 4C) is identical with the ratio of relative amount of total PDE4D, PDE$_{tot}$, and relative amount of total tubulin, tubulin$_{total}$.

Activity assay and inhibition of phosphodiesterase activity

[0061] An IMAP FP-Assay was used for the determination of phosphodiesterase activity. The phosphodiesterase activity of the core contruct and PDE4D3 was measured using the HEFP Phosphodiesterase Assay Kit (Molecular Devices). 2 µl of PDE4D3 or PDE4D core construct, 2 µl of cAMP (to a final concentration of 40 nM) and 1 µl of test substance or carrier were incubated for 45 min on a shaker. 12 µl of Binding Solution provided by the kit (with beads diluted 1:320) were added, and the reaction mixture incubated on a shaker for 2 hours. Fluorescence polarisation of the samples was measured in a Packard BioScience Fusion a-FP HT using as an emission filter a Polarizer 535, and as an excitation filter, Fluorescein 485/20. Inhibition of the phosphodiesterase activity of the PDE4D core construct was determined using Rolipram as inhibitor, and using PDE4D core construct at 30 ng/ml.

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11

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Leu Leu Asp Asn Tyr Ser Asp Arg Ile Gln Val Leu Gln Asn Met Val
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12

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            515   520   525

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Pro Leu Trp Glu Thr Trp Ala Asp Leu Val His Pro Asp Ala Gln Asp

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Ile Leu Asp Thr Leu Glu Asp Asn Arg Glu Trp Tyr Gln Ser Thr Ile

            565   570   575

Pro Gln Ser Pro Ser Pro Ala Pro Asp Asp Pro Glu Glu Gly Arg Gln

            580   585   590

Gly Gln Thr Glu Lys Phe Gln Phe Glu Leu Thr Leu Glu Glu Asp Gly

            595   600   605

Glu Ser Asp Thr Glu Lys Asp Ser Gly Ser Gln Val Glu Glu Asp Thr

            610   615   620

Ser Cys Ser Asp Ser Lys Thr Leu Cys Thr Gln Asp Ser Glu Ser Thr

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Leu Leu Leu Asp Asn Tyr Ser Asp Arg Ile Gln Val Leu Gln Asn Met

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Thr Ser Ala Gly Arg Ser Pro Leu Asp Pro Met Thr Ser Pro Gly Ser
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Gly Leu Ile Leu Gln Ala Asn Phe Val His Ser Gln Arg Arg Glu Ser
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Ser Pro Met Cys Asn Gln Pro Ser Ile Asn Lys Ala Thr Ile Thr Glu
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Cys Leu Asp Gln Leu Glu Thr Leu Gln Thr Arg His Ser Val Ser Glu
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Met Ala Ser Asn Lys Phe Lys Arg Met Leu Asn Arg Glu Leu Thr His
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Leu Ser Glu Met Ser Arg Ser Gly Asn Gln Val Ser Glu Phe Ile Ser
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Asn Thr Phe Leu Asp Lys Gln His Glu Val Glu Ile Pro Ser Pro Thr
            275                 280                 285

Gln Lys Glu Lys Glu Lys Lys Lys Arg Pro Met Ser Gln Ile Ser Gly
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Val Lys Lys Leu Met His Ser Ser Ser Leu Thr Asn Ser Ser Ile Pro
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Tyr Leu Met Thr Leu Glu Asp His Tyr His Ala Asp Val Ala Tyr His
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Asn Asn Ile His Ala Ala Asp Val Val Gln Ser Thr His Val Leu Leu
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    50                  55                  60

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                            100                     105                     110

              Leu Arg Thr Val Arg Asn Asn Phe Ala Ala Leu Thr Asn Leu Gln Asp

                            115                     120                     125

              Arg Ala Pro Ser Lys Arg Ser Pro Met Cys Asn Gln Pro Ser Ile Asn

                      130                     135                     140

              Lys Ala Thr Ile Thr Glu Glu Ala Tyr Gln Lys Leu Ala Ser Glu Thr

              145                     150                     155                     160

              Leu Glu Glu Leu Asp Trp Cys Leu Asp Gln Leu Glu Thr Leu Gln Thr

                            165                     170                     175

              Arg His Ser Val Ser Glu Met Ala Ser Asn Lys Phe Lys Arg Met Leu

                            180                     185                     190

              Asn Arg Glu Leu Thr His Leu Ser Glu Met Ser Arg Ser Gly Asn Gln

                            195                     200                     205

              Val Ser Glu Phe Ile Ser Asn Thr Phe Leu Asp Lys Gln His Glu Val

                      210                     215                     220

              Glu Ile Pro Ser Pro Thr Gln Lys Glu Lys Glu Lys Lys Lys Arg Pro

              225                     230                     235                     240

              Met Ser Gln Ile Ser Gly Val Lys Lys Leu Met His Ser Ser Ser Leu

                            245                     250                     255

              Thr Asn Ser Ser Ile Pro Arg Phe Gly Val Lys Thr Glu Gln Glu Asp
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            290   295   300

    His Thr Ile Phe Gln Glu Arg Asp Leu Leu Lys Thr Phe Lys Ile Pro

    305   310   315   320

    Val Asp Thr Leu Ile Thr Tyr Leu Met Thr Leu Glu Asp His Tyr His

                             325   330   335

    Ala Asp Val Ala Tyr His Asn Asn Ile His Ala Ala Asp Val Val Gln

                             340   345   350

    Ser Thr His Val Leu Leu Ser Thr Pro Ala Leu Glu Ala Val Phe Thr

                             355   360   365

    Asp Leu Glu Ile Leu Ala Ala Ile Phe Ala Ser Ala Ile His Asp Val

            370   375   380

    Asp His Pro Gly Val Ser Asn Gln Phe Leu Ile Asn Thr Asn Ser Glu

    385   390   395   400

    Leu Ala Leu Met Tyr Asn Asp Ser Ser Val Leu Glu Asn His His Leu

                             405   410   415

    Ala Val Gly Phe Lys Leu Leu Gln Glu Glu Asn Cys Asp Ile Phe Gln

                             420   425   430

    Asn Leu Thr Lys Lys Gln Arg Gln Ser Leu Arg Lys Met Val Ile Asp

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    Ile Val Leu Ala Thr Asp Met Ser Lys His Met Asn Leu Leu Ala Asp

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                465                     470                     475                 480
        Leu Leu Asp Asn Tyr Ser Asp Arg Ile Gln Val Leu Gln Asn Met Val

                            485                     490                     495
        His Cys Ala Asp Leu Ser Asn Pro Thr Lys Pro Leu Gln Leu Tyr Arg

                    500                     505                     510
        Gln Trp Thr Asp Arg Ile Met Glu Glu Phe Phe Arg Gln Gly Asp Arg

                    515                     520                     525
        Glu Arg Glu Arg Gly Met Glu Ile Ser Pro Met Cys Asp Lys His Asn

                530                     535                     540
        Ala Ser Val Glu Lys Ser Gln Val Gly Phe Ile Asp Tyr Ile Val His

                545                     550                     555                 560
        Pro Leu Trp Glu Thr Trp Ala Asp Leu Val His Pro Asp Ala Gln Asp

                            565                     570                     575
        Ile Leu Asp Thr Leu Glu Asp Asn Arg Glu Trp Tyr Gln Ser Thr Ile

                    580                     585                     590
        Pro Gln Ser Pro Ser Pro Ala Pro Asp Asp Pro Glu Glu Gly Arg Gln

                    595                     600                     605
        Gly Gln Thr Glu Lys Phe Gln Phe Glu Leu Thr Leu Glu Glu Asp Gly

                610                     615                     620
        Glu Ser Asp Thr Glu Lys Asp Ser Gly Ser Gln Val Glu Glu Asp Thr

                625                     630                     635                 640
        Ser Cys Ser Asp Ser Lys Thr Leu Cys Thr Gln Asp Ser Glu Ser Thr
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645   650   655

Glu Ile Pro Leu Asp Glu Gln Val Glu Glu Glu Ala Val Gly Glu Glu

660   665   670

Glu Glu Ser Gln Pro Glu Ala Cys Val Ile Asp Asp Arg Ser Pro Asp

675   680   685

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Glu Glu Thr Leu His Ser Ser Asn Glu Glu Glu Asp Pro Phe Arg Gly
                    20   25   30

Met Glu Pro Tyr Leu Val Arg Arg Leu Ser Cys Arg Asn Ile Gln Leu
                    35   40   45

Pro Pro Leu Ala Phe Arg Gln Leu Glu Gln Ala Asp Leu Lys Ser Glu
        50   55   60

Ser Glu Asn Ile Gln Arg Pro Thr Ser Leu Pro Leu Lys Ile Leu Pro
65   70   75   80

Leu Ile Ala Ile Thr Ser Ala Glu Ser Ser Gly Lys Leu Phe Asp Val
                85   90   95

Asp Asn Gly Thr Ser Ala Gly Arg Ser Pro Leu Asp Pro Met Thr Ser
            100   105   110

Pro Gly Ser Gly Leu Ile Leu Gln Ala Asn Phe Val His Ser Gln Arg
            115   120   125

Arg Glu Ser Phe Leu Tyr Arg Ser Asp Ser Asp Tyr Asp Leu Ser Pro
        130   135   140

Lys Ser Met Ser Arg Asn Ser Ser Ile Ala Ser Asp Ile His Gly Asp
145   150   155   160

Asp Leu Ile Val Thr Pro Phe Ala Gln Val Leu Ala Ser Leu Arg Thr
                165   170   175

Val Arg Asn Asn Phe Ala Ala Leu Thr Asn Leu Gln Asp Arg Ala Pro
                180   185   190

Ser Lys Arg Ser Pro Met Cys Asn Gln Pro Ser Ile Asn Lys Ala Thr
            195   200   205

Ile Thr Glu Glu Ala Tyr Gln Lys Leu Ala Ser Glu Thr Leu Glu Glu
            210   215   220

Leu Asp Trp Cys Leu Asp Gln Leu Glu Thr Leu Gln Thr Arg His Ser
225   230   235   240

Val Ser Glu Met Ala Ser Asn Lys Phe Lys Arg Met Leu Asn Arg Glu
                    245   250   255

Leu Thr His Leu Ser Glu Met Ser Arg Ser Gly Asn Gln Val Ser Glu
                    260   265   270

Phe Ile Ser Asn Thr Phe Leu Asp Lys Gln His Glu Val Glu Ile Pro
                    275   280   285

Ser Pro Thr Gln Lys Glu Lys Glu Lys Lys Lys Arg Pro Met Ser Gln
                    290   295   300

Ile Ser Gly Val Lys Lys Leu Met His Ser Ser Ser Leu Thr Asn Ser
305   310   315   320

Ser Ile Pro Arg Phe Gly Val Lys Thr Glu Gln Glu Asp Val Leu Ala
                    325   330   335

Lys Glu Leu Glu Asp Val Asn Lys Trp Gly Leu His Val Phe Arg Ile
                    340   345   350

Ala Glu Leu Ser Gly Asn Arg Pro Leu Thr Val Ile Met His Thr Ile
                    355   360   365

Phe Gln Glu Arg Asp Leu Leu Lys Thr Phe Lys Ile Pro Val Asp Thr
                    370   375   380

Leu Ile Thr Tyr Leu Met Thr Leu Glu Asp His Tyr His Ala Asp Val
385   390   395   400

Ala Tyr His Asn Asn Ile His Ala Ala Asp Val Val Gln Ser Thr His
                    405   410   415

Val Leu Leu Ser Thr Pro Ala Leu Glu Ala Val Phe Thr Asp Leu Glu
                    420   425   430

Ile Leu Ala Ala Ile Phe Ala Ser Ala Ile His Asp Val Asp His Pro
      435   440   445

Gly Val Ser Asn Gln Phe Leu Ile Asn Thr Asn Ser Glu Leu Ala Leu
      450   455   460

Met Tyr Asn Asp Ser Ser Val Leu Glu Asn His His Leu Ala Val Gly
465   470   475   480

Phe Lys Leu Leu Gln Glu Glu Asn Cys Asp Ile Phe Gln Asn Leu Thr
          485   490   495

Lys Lys Gln Arg Gln Ser Leu Arg Lys Met Val Ile Asp Ile Val Leu
      500   505   510

Ala Thr Asp Met Ser Lys His Met Asn Leu Leu Ala Asp Leu Lys Thr
      515   520   525

Met Val Glu Thr Lys Lys Val Thr Ser Ser Gly Val Leu Leu Leu Asp
      530   535   540

Asn Tyr Ser Asp Arg Ile Gln Val Leu Gln Asn Met Val His Cys Ala
545   550   555   560

Asp Leu Ser Asn Pro Thr Lys Pro Leu Gln Leu Tyr Arg Gln Trp Thr
          565   570   575

Asp Arg Ile Met Glu Glu Phe Phe Arg Gln Gly Asp Arg Glu Arg Glu
          580   585   590

Arg Gly Met Glu Ile Ser Pro Met Cys Asp Lys His Asn Ala Ser Val
      595   600   605

Glu Lys Ser Gln Val Gly Phe Ile Asp Tyr Ile Val His Pro Leu Trp
      610   615   620

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Glu Thr Trp Ala Asp Leu Val His Pro Asp Ala Gln Asp Ile Leu Asp

625                 630                 635                 640

Thr Leu Glu Asp Asn Arg Glu Trp Tyr Gln Ser Thr Ile Pro Gln Ser

                645                 650                 655

Pro Ser Pro Ala Pro Asp Asp Pro Glu Glu Gly Arg Gln Gly Gln Thr

                660                 665                 670

Glu Lys Phe Gln Phe Glu Leu Thr Leu Glu Glu Asp Gly Glu Ser Asp

                675                 680                 685

Thr Glu Lys Asp Ser Gly Ser Gln Val Glu Glu Asp Thr Ser Cys Ser

        690                 695                 700

Asp Ser Lys Thr Leu Cys Thr Gln Asp Ser Glu Ser Thr Glu Ile Pro

705                 710                 715                 720

Leu Asp Glu Gln Val Glu Glu Glu Ala Val Gly Glu Glu Glu Glu Ser

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Gln Pro Glu Ala Cys Val Ile Asp Asp Arg Ser Pro Asp Thr

                740                 745                 750
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Thr Ala Glu Ala Lys Leu Phe Asp Val Asp Asn Gly Thr Ser Ala Gly
            20                  25                  30

Arg Ser Pro Leu Asp Pro Met Thr Ser Pro Gly Ser Gly Leu Ile Leu
            35                  40                  45

Gln Ala Asn Phe Val His Ser Gln Arg Arg Glu Ser Phe Leu Tyr Arg
        50                  55                  60

Ser Asp Ser Asp Tyr Asp Leu Ser Pro Lys Ser Met Ser Arg Asn Ser
65                  70                  75                  80

Ser Ile Ala Ser Asp Ile His Gly Asp Asp Leu Ile Val Thr Pro Phe
                85                  90                  95

Ala Gln Val Leu Ala Ser Leu Arg Thr Val Arg Asn Asn Phe Ala Ala
            100                 105                 110

Leu Thr Asn Leu Gln Asp Arg Ala Pro Ser Lys Arg Ser Pro Met Cys
            115                 120                 125

Asn Gln Pro Ser Ile Asn Lys Ala Thr Ile Thr Glu Glu Ala Tyr Gln
        130                 135                 140

Lys Leu Ala Ser Glu Thr Leu Glu Glu Leu Asp Trp Cys Leu Asp Gln
145                 150                 155                 160

Leu Glu Thr Leu Gln Thr Arg His Ser Val Ser Glu Met Ala Ser Asn
                165                 170                 175
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Lys Phe Lys Arg Met Leu Asn Arg Glu Leu Thr His Leu Ser Glu Met
                180                 185                 190

Ser Arg Ser Gly Asn Gln Val Ser Glu Phe Ile Ser Asn Thr Phe Leu
            195                 200                 205

Asp Lys Gln His Glu Val Glu Ile Pro Ser Pro Thr Gln Lys Glu Lys
        210                 215                 220

Glu Lys Lys Lys Arg Pro Met Ser Gln Ile Ser Gly Val Lys Lys Leu
225                 230                 235                 240

Met His Ser Ser Ser Leu Thr Asn Ser Ser Ile Pro Arg Phe Gly Val
            245                 250                 255

Lys Thr Glu Gln Glu Asp Val Leu Ala Lys Glu Leu Glu Asp Val Asn
                260                 265                 270

Lys Trp Gly Leu His Val Phe Arg Ile Ala Glu Leu Ser Gly Asn Arg
            275                 280                 285

Pro Leu Thr Val Ile Met His Thr Ile Phe Gln Glu Arg Asp Leu Leu
        290                 295                 300

Lys Thr Phe Lys Ile Pro Val Asp Thr Leu Ile Thr Tyr Leu Met Thr
305                 310                 315                 320

Leu Glu Asp His Tyr His Ala Asp Val Ala Tyr His Asn Asn Ile His
            325                 330                 335

Ala Ala Asp Val Val Gln Ser Thr His Val Leu Leu Ser Thr Pro Ala
            340                 345                 350

Leu Glu Ala Val Phe Thr Asp Leu Glu Ile Leu Ala Ala Ile Phe Ala
            355                 360                 365
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Ser Ala Ile His Asp Val Asp His Pro Gly Val Ser Asn Gln Phe Leu
        370   375   380

Ile Asn Thr Asn Ser Glu Leu Ala Leu Met Tyr Asn Asp Ser Ser Val
385   390   395   400

Leu Glu Asn His His Leu Ala Val Gly Phe Lys Leu Leu Gln Glu Glu
                405   410   415

Asn Cys Asp Ile Phe Gln Asn Leu Thr Lys Lys Gln Arg Gln Ser Leu
        420   425   430

Arg Lys Met Val Ile Asp Ile Val Leu Ala Thr Asp Met Ser Lys His
        435   440   445

Met Asn Leu Leu Ala Asp Leu Lys Thr Met Val Glu Thr Lys Lys Val
        450   455   460

Thr Ser Ser Gly Val Leu Leu Leu Asp Asn Tyr Ser Asp Arg Ile Gln
465   470   475   480

Val Leu Gln Asn Met Val His Cys Ala Asp Leu Ser Asn Pro Thr Lys
                485   490   495

Pro Leu Gln Leu Tyr Arg Gln Trp Thr Asp Arg Ile Met Glu Glu Phe
        500   505   510

Phe Arg Gln Gly Asp Arg Glu Arg Glu Arg Gly Met Glu Ile Ser Pro
        515   520   525

Met Cys Asp Lys His Asn Ala Ser Val Glu Lys Ser Gln Val Gly Phe
        530   535   540

Ile Asp Tyr Ile Val His Pro Leu Trp Glu Thr Trp Ala Asp Leu Val
545   550   555   560

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His Pro Asp Ala Gln Asp Ile Leu Asp Thr Leu Glu Asp Asn Arg Glu
                    565             570             575

Trp Tyr Gln Ser Thr Ile Pro Gln Ser Pro Ser Pro Ala Pro Asp Asp
                    580             585             590

Pro Glu Glu Gly Arg Gln Gly Gln Thr Glu Lys Phe Gln Phe Glu Leu
                595             600             605

Thr Leu Glu Glu Asp Gly Glu Ser Asp Thr Glu Lys Asp Ser Gly Ser
                610             615             620

Gln Val Glu Glu Asp Thr Ser Cys Ser Asp Ser Lys Thr Leu Cys Thr
625             630             635             640

Gln Asp Ser Glu Ser Thr Glu Ile Pro Leu Asp Glu Gln Val Glu Glu
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Glu Ala Val Gly Glu Glu Glu Glu Ser Gln Pro Glu Ala Cys Val Ile
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Asp Asp Arg Ser Pro Asp Thr
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Gly Ser Asp Ser Ala Gly Gly Ala Thr Leu Lys Ala Pro Lys His Leu
                20   25   30

Trp Arg His Glu Gln His His Gln Tyr Pro Leu Arg Gln Pro Gln Phe
                35   40   45

Arg Leu Leu His Pro His His His Leu Pro Pro Pro Pro Pro Pro Ser
            50   55   60

Pro Gln Pro Gln Pro Gln Cys Pro Leu Gln Pro Pro Pro Pro Pro Pro
65   70   75   80

Leu Pro Pro Pro Pro Pro Pro Gly Ala Ala Arg Gly Arg Tyr Ala
                85   90   95

Ser Ser Gly Ala Thr Gly Arg Val Arg His Arg Gly Tyr Ser Asp Thr
            100   105   110

Glu Arg Tyr Leu Tyr Cys Arg Ala Met Asp Arg Thr Ser Tyr Ala Val
            115   120   125

Glu Thr Gly His Arg Pro Gly Leu Lys Lys Ser Arg Met Ser Trp Pro
        130   135   140

Ser Ser Phe Gln Gly Leu Arg Arg Phe Asp Val Asp Asn Gly Thr Ser
145   150   155   160

Ala Gly Arg Ser Pro Leu Asp Pro Met Thr Ser Pro Gly Ser Gly Leu
                165   170   175

Ile Leu Gln Ala Asn Phe Val His Ser Gln Arg Arg Glu Ser Phe Leu
                180   185   190

Tyr Arg Ser Asp Ser Asp Tyr Asp Leu Ser Pro Lys Ser Met Ser Arg
            195   200   205

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Asn Ser Ser Ile Ala Ser Asp Ile His Gly Asp Asp Leu Ile Val Thr
        210             215             220

Pro Phe Ala Gln Val Leu Ala Ser Leu Arg Thr Val Arg Asn Asn Phe
225             230             235             240

Ala Ala Leu Thr Asn Leu Gln Asp Arg Ala Pro Ser Lys Arg Ser Pro
                245             250             255

Met Cys Asn Gln Pro Ser Ile Asn Lys Ala Thr Ile Thr Glu Glu Ala
                260             265             270

Tyr Gln Lys Leu Ala Ser Glu Thr Leu Glu Glu Leu Asp Trp Cys Leu
        275             280             285

Asp Gln Leu Glu Thr Leu Gln Thr Arg His Ser Val Ser Glu Met Ala
        290             295             300

Ser Asn Lys Phe Lys Arg Met Leu Asn Arg Glu Leu Thr His Leu Ser
305             310             315             320

Glu Met Ser Arg Ser Gly Asn Gln Val Ser Glu Phe Ile Ser Asn Thr
                325             330             335

Phe Leu Asp Lys Gln His Glu Val Glu Ile Pro Ser Pro Thr Gln Lys
                340             345             350

Glu Lys Glu Lys Lys Lys Arg Pro Met Ser Gln Ile Ser Gly Val Lys
        355             360             365

Lys Leu Met His Ser Ser Ser Leu Thr Asn Ser Ser Ile Pro Arg Phe
        370             375             380

Gly Val Lys Thr Glu Gln Glu Asp Val Leu Ala Lys Glu Leu Glu Asp
385             390             395             400
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42

Val Asn Lys Trp Gly Leu His Val Phe Arg Ile Ala Glu Leu Ser Gly
                 405   410   415

Asn Arg Pro Leu Thr Val Ile Met His Thr Ile Phe Gln Glu Arg Asp
                 420   425   430

Leu Leu Lys Thr Phe Lys Ile Pro Val Asp Thr Leu Ile Thr Tyr Leu
                 435   440   445

Met Thr Leu Glu Asp His Tyr His Ala Asp Val Ala Tyr His Asn Asn
             450   455   460

Ile His Ala Ala Asp Val Val Gln Ser Thr His Val Leu Leu Ser Thr
465   470   475   480

Pro Ala Leu Glu Ala Val Phe Thr Asp Leu Glu Ile Leu Ala Ala Ile
                 485   490   495

Phe Ala Ser Ala Ile His Asp Val Asp His Pro Gly Val Ser Asn Gln
                 500   505   510

Phe Leu Ile Asn Thr Asn Ser Glu Leu Ala Leu Met Tyr Asn Asp Ser
             515   520   525

Ser Val Leu Glu Asn His His Leu Ala Val Gly Phe Lys Leu Leu Gln
             530   535   540

Glu Glu Asn Cys Asp Ile Phe Gln Asn Leu Thr Lys Lys Gln Arg Gln
545   550   555   560

Ser Leu Arg Lys Met Val Ile Asp Ile Val Leu Ala Thr Asp Met Ser
                 565   570   575

Lys His Met Asn Leu Leu Ala Asp Leu Lys Thr Met Val Glu Thr Lys
                 580   585   590

Lys Val Thr Ser Ser Gly Val Leu Leu Leu Asp Asn Tyr Ser Asp Arg
       595   600   605

Ile Gln Val Leu Gln Asn Met Val His Cys Ala Asp Leu Ser Asn Pro
       610   615   620

Thr Lys Pro Leu Gln Leu Tyr Arg Gln Trp Thr Asp Arg Ile Met Glu
625   630   635   640

Glu Phe Phe Arg Gln Gly Asp Arg Glu Arg Glu Arg Gly Met Glu Ile
           645   650   655

Ser Pro Met Cys Asp Lys His Asn Ala Ser Val Glu Lys Ser Gln Val
       660   665   670

Gly Phe Ile Asp Tyr Ile Val His Pro Leu Trp Glu Thr Trp Ala Asp
       675   680   685

Leu Val His Pro Asp Ala Gln Asp Ile Leu Asp Thr Leu Glu Asp Asn
       690   695   700

Arg Glu Trp Tyr Gln Ser Thr Ile Pro Gln Ser Pro Ser Pro Ala Pro
705   710   715   720

Asp Asp Pro Glu Glu Gly Arg Gln Gly Gln Thr Glu Lys Phe Gln Phe
           725   730   735

Glu Leu Thr Leu Glu Glu Asp Gly Glu Ser Asp Thr Glu Lys Asp Ser
           740   745   750

Gly Ser Gln Val Glu Glu Asp Thr Ser Cys Ser Asp Ser Lys Thr Leu
       755   760   765

Cys Thr Gln Asp Ser Glu Ser Thr Glu Ile Pro Leu Asp Glu Gln Val
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Glu Glu Glu Ala Val Gly Glu Glu Glu Glu Ser Gln Pro Glu Ala Cys

785   790   795   800

Val Ile Asp Asp Arg Ser Pro Asp Thr

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            35                  40                  45

Ser Gln Arg Arg Glu Ser Phe Leu Tyr Arg Ser Asp Ser Asp Tyr Asp
        50                  55                  60

Leu Ser Pro Lys Ser Met Ser Arg Asn Ser Ser Ile Ala Ser Asp Ile
65                  70                  75                  80

His Gly Asp Asp Leu Ile Val Thr Pro Phe Ala Gln Val Leu Ala Ser
                85                  90                  95
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Leu Arg Thr Val Arg Asn Asn Phe Ala Ala Leu Thr Asn Leu Gln Asp
100   105   110

Arg Ala Pro Ser Lys Arg Ser Pro Met Cys Asn Gln Pro Ser Ile Asn
115   120   125

Lys Ala Thr Ile Thr Glu Glu Ala Tyr Gln Lys Leu Ala Ser Glu Thr
130   135   140

Leu Glu Glu Leu Asp Trp Cys Leu Asp Gln Leu Glu Thr Leu Gln Thr
145   150   155   160

Arg His Ser Val Ser Glu Met Ala Ser Asn Lys Phe Lys Arg Met Leu
165   170   175

Asn Arg Glu Leu Thr His Leu Ser Glu Met Ser Arg Ser Gly Asn Gln
180   185   190

Val Ser Glu Phe Ile Ser Asn Thr Phe Leu Asp Lys Gln His Glu Val
195   200   205

Glu Ile Pro Ser Pro Thr Gln Lys Glu Lys Glu Lys Lys Lys Arg Pro
210   215   220

Met Ser Gln Ile Ser Gly Val Lys Lys Leu Met His Ser Ser Ser Leu
225   230   235   240

Thr Asn Ser Ser Ile Pro Arg Phe Gly Val Lys Thr Glu Gln Glu Asp
245   250   255

Val Leu Ala Lys Glu Leu Glu Asp Val Asn Lys Trp Gly Leu His Val
260   265   270

Phe Arg Ile Ala Glu Leu Ser Gly Asn Arg Pro Leu Thr Val Ile Met
275   280   285

His Thr Ile Phe Gln Glu Arg Asp Leu Leu Lys Thr Phe Lys Ile Pro
    290   295   300

Val Asp Thr Leu Ile Thr Tyr Leu Met Thr Leu Glu Asp His Tyr His
305   310   315   320

Ala Asp Val Ala Tyr His Asn Asn Ile His Ala Ala Asp Val Val Gln
                325   330   335

Ser Thr His Val Leu Leu Ser Thr Pro Ala Leu Glu Ala Val Phe Thr
                340   345   350

Asp Leu Glu Ile Leu Ala Ala Ile Phe Ala Ser Ala Ile His Asp Val
            355   360   365

Asp His Pro Gly Val Ser Asn Gln Phe Leu Ile Asn Thr Asn Ser Glu
    370   375   380

Leu Ala Leu Met Tyr Asn Asp Ser Ser Val Leu Glu Asn His His Leu
385   390   395   400

Ala Val Gly Phe Lys Leu Leu Gln Glu Glu Asn Cys Asp Ile Phe Gln
                405   410   415

Asn Leu Thr Lys Lys Gln Arg Gln Ser Leu Arg Lys Met Val Ile Asp
            420   425   430

Ile Val Leu Ala Thr Asp Met Ser Lys His Met Asn Leu Leu Ala Asp
            435   440   445

Leu Lys Thr Met Val Glu Thr Lys Lys Val Thr Ser Ser Gly Val Leu
        450   455   460

Leu Leu Asp Asn Tyr Ser Asp Arg Ile Gln Val Leu Gln Asn Met Val
465   470   475   480

His Cys Ala Asp Leu Ser Asn Pro Thr Lys Pro Leu Gln Leu Tyr Arg
                        485   490   495

Gln Trp Thr Asp Arg Ile Met Glu Glu Phe Phe Arg Gln Gly Asp Arg
                    500   505   510

Glu Arg Glu Arg Gly Met Glu Ile Ser Pro Met Cys Asp Lys His Asn
                 515   520   525

Ala Ser Val Glu Lys Ser Gln Val Gly Phe Ile Asp Tyr Ile Val His
                 530   535   540

Pro Leu Trp Glu Thr Trp Ala Asp Leu Val His Pro Asp Ala Gln Asp
545   550   555   560

Ile Leu Asp Thr Leu Glu Asp Asn Arg Glu Trp Tyr Gln Ser Thr Ile
                    565   570   575

Pro Gln Ser Pro Ser Pro Ala Pro Asp Asp Pro Glu Glu Gly Arg Gln
                    580   585   590

Gly Gln Thr Glu Lys Phe Gln Phe Glu Leu Thr Leu Glu Glu Asp Gly
                 595   600   605

Glu Ser Asp Thr Glu Lys Asp Ser Gly Ser Gln Val Glu Glu Asp Thr
                 610   615   620

Ser Cys Ser Asp Ser Lys Thr Leu Cys Thr Gln Asp Ser Glu Ser Thr
625   630   635   640

Glu Ile Pro Leu Asp Glu Gln Val Glu Glu Glu Ala Val Gly Glu Glu
                    645   650   655

Glu Glu Ser Gln Pro Glu Ala Cys Val Ile Asp Asp Arg Ser Pro Asp
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Thr

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Met Phe Asp Val Asp Asn Gly Thr Ser Ala Gly Arg Ser Pro Leu Asp
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Pro Met Thr Ser Pro Gly Ser Gly Leu Ile Leu Gln Ala Asn Phe Val
            20              25              30

His Ser Gln Arg Arg Glu Ser Phe Leu Tyr Arg Ser Asp Ser Asp Tyr
            35              40              45

Asp Leu Ser Pro Lys Ser Met Ser Arg Asn Ser Ser Ile Ala Ser Asp
            50              55              60

Ile His Gly Asp Asp Leu Ile Val Thr Pro Phe Ala Gln Val Leu Ala
65              70              75              80

Ser Leu Arg Thr Val Arg Asn Asn Phe Ala Ala Leu Thr Asn Leu Gln
            85              90              95

Asp Arg Ala Pro Ser Lys Arg Ser Pro Met Cys Asn Gln Pro Ser Ile
            100             105             110

Asn Lys Ala Thr Ile Thr Glu Glu Ala Tyr Gln Lys Leu Ala Ser Glu
            115             120             125
```

Thr Leu Glu Glu Leu Asp Trp Cys Leu Asp Gln Leu Glu Thr Leu Gln

     130   135   140

Thr Arg His Ser Val Ser Glu Met Ala Ser Asn Lys Phe Lys Arg Met

145   150   155   160

Leu Asn Arg Glu Leu Thr His Leu Ser Glu Met Ser Arg Ser Gly Asn

          165   170   175

Gln Val Ser Glu Phe Ile Ser Asn Thr Phe Leu Asp Lys Gln His Glu

          180   185   190

Val Glu Ile Pro Ser Pro Thr Gln Lys Glu Lys Glu Lys Lys Lys Arg

          195   200   205

Pro Met Ser Gln Ile Ser Gly Val Lys Lys Leu Met His Ser Ser Ser

          210   215   220

Leu Thr Asn Ser Ser Ile Pro Arg Phe Gly Val Lys Thr Glu Gln Glu

225   230   235   240

Asp Val Leu Ala Lys Glu Leu Glu Asp Val Asn Lys Trp Gly Leu His

          245   250   255

Val Phe Arg Ile Ala Glu Leu Ser Gly Asn Arg Pro Leu Thr Val Ile

          260   265   270

Met His Thr Ile Phe Gln Glu Arg Asp Leu Leu Lys Thr Phe Lys Ile

          275   280   285

Pro Val Asp Thr Leu Ile Thr Tyr Leu Met Thr Leu Glu Asp His Tyr

          290   295   300

His Ala Asp Val Ala Tyr His Asn Asn Ile His Ala Ala Asp Val Val

305   310   315   320

```
Gln Ser Thr His Val Leu Leu Ser Thr Pro Ala Leu Glu Ala Val Phe
            325             330             335

Thr Asp Leu Glu Ile Leu Ala Ala Ile Phe Ala Ser Ala Ile His Asp
            340             345             350

Val Asp His Pro Gly Val Ser Asn Gln Phe Leu Ile Asn Thr Asn Ser
            355             360             365

Glu Leu Ala Leu Met Tyr Asn Asp Ser Ser Val Leu Glu Asn His His
    370             375             380

Leu Ala Val Gly Phe Lys Leu Leu Gln Glu Glu Asn Cys Asp Ile Phe
385             390             395             400

Gln Asn Leu Thr Lys Lys Gln Arg Gln Ser Leu Arg Lys Met Val Ile
            405             410             415

Asp Ile Val Leu Ala Thr Asp Met Ser Lys His Met Asn Leu Leu Ala
            420             425             430

Asp Leu Lys Thr Met Val Glu Thr Lys Lys Val Thr Ser Ser Gly Val
            435             440             445

Leu Leu Leu Asp Asn Tyr Ser Asp Arg Ile Gln Val Leu Gln Asn Met
    450             455             460

Val His Cys Ala Asp Leu Ser Asn Pro Thr Lys Pro Leu Gln Leu Tyr
465             470             475             480

Arg Gln Trp Thr Asp Arg Ile Met Glu Glu Phe Phe Arg Gln Gly Asp
            485             490             495

Arg Glu Arg Glu Arg Gly Met Glu Ile Ser Pro Met Cys Asp Lys His
            500             505             510
```

```
Asn Ala Ser Val Glu Lys Ser Gln Val Gly Phe Ile Asp Tyr Ile Val
        515                 520                 525

His Pro Leu Trp Glu Thr Trp Ala Asp Leu Val His Pro Asp Ala Gln
        530                 535                 540

Asp Ile Leu Asp Thr Leu Glu Asp Asn Arg Glu Trp Tyr Gln Ser Thr
545                 550                 555                 560

Ile Pro Gln Ser Pro Ser Pro Ala Pro Asp Asp Pro Glu Glu Gly Arg
                565                 570                 575

Gln Gly Gln Thr Glu Lys Phe Gln Phe Glu Leu Thr Leu Glu Glu Asp
                580                 585                 590

Gly Glu Ser Asp Thr Glu Lys Asp Ser Gly Ser Gln Val Glu Glu Asp
        595                 600                 605

Thr Ser Cys Ser Asp Ser Lys Thr Leu Cys Thr Gln Asp Ser Glu Ser
        610                 615                 620

Thr Glu Ile Pro Leu Asp Glu Gln Val Glu Glu Glu Ala Val Gly Glu
625                 630                 635                 640

Glu Glu Glu Ser Gln Pro Glu Ala Cys Val Ile Asp Asp Arg Ser Pro
                645                 650                 655

Asp Thr His His His His His His
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Met Ala Gln Gln Thr Ser Pro Asp Thr Leu Thr Val Pro Glu Val Asp
1               5                   10                  15

Asn Pro His Cys Pro Asn Pro Trp Leu Asn Glu Asp Leu Val Lys Ser
            20                  25                  30

Leu Arg Glu Asn Leu Leu Gln His Glu Lys Ser Lys Thr Ala Arg Lys
            35                  40                  45

Ser Val Ser Pro Lys Leu Ser Pro Val Ile Ser Pro Arg Asn Ser Pro
            50                  55                  60

Arg Leu Leu Arg Arg Met Leu Leu Ser Ser Asn Ile Pro Lys Gln Arg
65                  70                  75                  80

Arg Phe Thr Val Ala His Thr Cys Lys Leu Phe Asp Val Asp Asn Gly
                85                  90                  95

Thr Ser Ala Gly Arg Ser Pro Leu Asp Pro Met Thr Ser Pro Gly Ser
                100                 105                 110

Gly Leu Ile Leu Gln Ala Asn Phe Val His Ser Gln Arg Arg Glu Ser
            115                 120                 125

Phe Leu Tyr Arg Ser Asp Ser Asp Tyr Asp Leu Ser Pro Lys Ser Met
        130                 135                 140
```

Ser Arg Asn Ser Ser Ile Ala Ser Asp Ile His Gly Asp Asp Leu Ile

145   150   155   160

Val Thr Pro Phe Ala Gln Val Leu Ala Ser Leu Arg Thr Val Arg Asn

          165   170   175

Asn Phe Ala Ala Leu Thr Asn Leu Gln Asp Arg Ala Pro Ser Lys Arg

          180   185   190

Ser Pro Met Cys Asn Gln Pro Ser Ile Asn Lys Ala Thr Ile Thr Glu

          195   200   205

Glu Ala Tyr Gln Lys Leu Ala Ser Glu Thr Leu Glu Glu Leu Asp Trp

          210   215   220

Cys Leu Asp Gln Leu Glu Thr Leu Gln Thr Arg His Ser Val Ser Glu

225   230   235   240

Met Ala Ser Asn Lys Phe Lys Arg Met Leu Asn Arg Glu Leu Thr His

          245   250   255

Leu Ser Glu Met Ser Arg Ser Gly Asn Gln Val Ser Glu Phe Ile Ser

          260   265   270

Asn Thr Phe Leu Asp Lys Gln His Glu Val Glu Ile Pro Ser Pro Thr

          275   280   285

Gln Lys Glu Lys Glu Lys Lys Lys Arg Pro Met Ser Gln Ile Ser Gly

          290   295   300

Val Lys Lys Leu Met His Ser Ser Ser Leu Thr Asn Ser Ser Ile Pro

305   310   315   320

Arg Phe Gly Val Lys Thr Glu Gln Glu Asp Val Leu Ala Lys Glu Leu

          325   330   335

```
Glu Asp Val Asn Lys Trp Gly Leu His Val Phe Arg Ile Ala Glu Leu
                340                 345                 350

Ser Gly Asn Arg Pro Leu Thr Val Ile Met His Thr Ile Phe Gln Glu
                355                 360                 365

Arg Asp Leu Leu Lys Thr Phe Lys Ile Pro Val Asp Thr Leu Ile Thr
       370                 375                 380

Tyr Leu Met Thr Leu Glu Asp His Tyr His Ala Asp Val Ala Tyr His
385                 390                 395                 400

Asn Asn Ile His Ala Ala Asp Val Val Gln Ser Thr His Val Leu Leu
                405                 410                 415

Ser Thr Pro Ala Leu Glu Ala Val Phe Thr Asp Leu Glu Ile Leu Ala
                420                 425                 430

Ala Ile Phe Ala Ser Ala Ile His Asp Val Asp His Pro Gly Val Ser
                435                 440                 445

Asn Gln Phe Leu Ile Asn Thr Asn Ser Glu Leu Ala Leu Met Tyr Asn
       450                 455                 460

Asp Ser Ser Val Leu Glu Asn His His Leu Ala Val Gly Phe Lys Leu
465                 470                 475                 480

Leu Gln Glu Glu Asn Cys Asp Ile Phe Gln Asn Leu Thr Lys Lys Gln
                485                 490                 495

Arg Gln Ser Leu Arg Lys Met Val Ile Asp Ile Val Leu Ala Thr Asp
                500                 505                 510

Met Ser Lys His Met Asn Leu Leu Ala Asp Leu Lys Thr Met Val Glu
                515                 520                 525
```

Thr Lys Lys Val Thr Ser Ser Gly Val Leu Leu Leu Asp Asn Tyr Ser
      530   535   540

Asp Arg Ile Gln Val Leu Gln Asn Met Val His Cys Ala Asp Leu Ser
545   550   555   560

Asn Pro Thr Lys Pro Leu Gln Leu Tyr Arg Gln Trp Thr Asp Arg Ile
             565   570   575

Met Glu Glu Phe Phe Arg Gln Gly Asp Arg Glu Arg Glu Arg Gly Met
             580   585   590

Glu Ile Ser Pro Met Cys Asp Lys His Asn Ala Ser Val Glu Lys Ser
             595   600   605

Gln Val Gly Phe Ile Asp Tyr Ile Val His Pro Leu Trp Glu Thr Trp
             610   615   620

Ala Asp Leu Val His Pro Asp Ala Gln Asp Ile Leu Asp Thr Leu Glu
625   630   635   640

Asp Asn Arg Glu Trp Tyr Gln Ser Thr Ile Pro Gln Ser Pro Ser Pro
             645   650   655

Ala Pro Asp Asp Pro Glu Glu Gly Arg Gln Gly Gln Thr Glu Lys Phe
             660   665   670

Gln Phe Glu Leu Thr Leu Glu Glu Asp Gly Glu Ser Asp Thr Glu Lys
             675   680   685

Asp Ser Gly Ser Gln Val Glu Glu Asp Thr Ser Cys Ser Asp Ser Lys
          690   695   700

Thr Leu Cys Thr Gln Asp Ser Glu Ser Thr Glu Ile Pro Leu Asp Glu
705   710   715   720

Gln Val Glu Glu Glu Ala Val Gly Glu Glu Glu Glu Ser Gln Pro Glu

725   730   735

Ala Cys Val Ile Asp Asp Arg Ser Pro Asp Thr

740   745

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Met Ala Phe Val Trp Asp Pro Leu Gly Ala Thr Val Pro Gly Pro Ser
1               5                   10                  15

Thr Arg Ala Lys Ser Arg Leu Arg Phe Ser Lys Ser Tyr Ser Lys Leu
            20                  25                  30

Phe Asp Val Asp Asn Gly Thr Ser Ala Gly Arg Ser Pro Leu Asp Pro
            35                  40                  45

Met Thr Ser Pro Gly Ser Gly Leu Ile Leu Gln Ala Asn Phe Val His
    50                  55                  60

Ser Gln Arg Arg Glu Ser Phe Leu Tyr Arg Ser Asp Ser Asp Tyr Asp
```

<pre>
        65   70   75   80

      Leu Ser Pro Lys Ser Met Ser Arg Asn Ser Ser Ile Ala Ser Asp Ile

                       85   90   95

      His Gly Asp Asp Leu Ile Val Thr Pro Phe Ala Gln Val Leu Ala Ser

                      100   105   110

      Leu Arg Thr Val Arg Asn Asn Phe Ala Ala Leu Thr Asn Leu Gln Asp

                      115   120   125

      Arg Ala Pro Ser Lys Arg Ser Pro Met Cys Asn Gln Pro Ser Ile Asn

                     130   135   140

      Lys Ala Thr Ile Thr Glu Glu Ala Tyr Gln Lys Leu Ala Ser Glu Thr

      145   150   155   160

      Leu Glu Glu Leu Asp Trp Cys Leu Asp Gln Leu Glu Thr Leu Gln Thr

                      165   170   175

      Arg His Ser Val Ser Glu Met Ala Ser Asn Lys Phe Lys Arg Met Leu

                      180   185   190

      Asn Arg Glu Leu Thr His Leu Ser Glu Met Ser Arg Ser Gly Asn Gln

                     195   200   205

      Val Ser Glu Phe Ile Ser Asn Thr Phe Leu Asp Lys Gln His Glu Val

                     210   215   220

      Glu Ile Pro Ser Pro Thr Gln Lys Glu Lys Glu Lys Lys Lys Arg Pro

      225   230   235   240

      Met Ser Gln Ile Ser Gly Val Lys Lys Leu Met His Ser Ser Ser Leu

                     245   250   255

      Thr Asn Ser Ser Ile Pro Arg Phe Gly Val Lys Thr Glu Gln Glu Asp
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```
            260                265                270

Val Leu Ala Lys Glu Leu Glu Asp Val Asn Lys Trp Gly Leu His Val

            275                280                285

Phe Arg Ile Ala Glu Leu Ser Gly Asn Arg Pro Leu Thr Val Ile Met

            290                295                300

His Thr Ile Phe Gln Glu Arg Asp Leu Leu Lys Thr Phe Lys Ile Pro

305             310                315                320

Val Asp Thr Leu Ile Thr Tyr Leu Met Thr Leu Glu Asp His Tyr His

                325                330                335

Ala Asp Val Ala Tyr His Asn Asn Ile His Ala Ala Asp Val Val Gln

                340                345                350

Ser Thr His Val Leu Leu Ser Thr Pro Ala Leu Glu Ala Val Phe Thr

                355                360                365

Asp Leu Glu Ile Leu Ala Ala Ile Phe Ala Ser Ala Ile His Asp Val

            370                375                380

Asp His Pro Gly Val Ser Asn Gln Phe Leu Ile Asn Thr Asn Ser Glu

385             390                395                400

Leu Ala Leu Met Tyr Asn Asp Ser Ser Val Leu Glu Asn His His Leu

                405                410                415

Ala Val Gly Phe Lys Leu Leu Gln Glu Glu Asn Cys Asp Ile Phe Gln

                420                425                430

Asn Leu Thr Lys Lys Gln Arg Gln Ser Leu Arg Lys Met Val Ile Asp

            435                440                445

Ile Val Leu Ala Thr Asp Met Ser Lys His Met Asn Leu Leu Ala Asp
```

```
           450                      455                      460
 5   Leu Lys Thr Met Val Glu Thr Lys Lys Val Thr Ser Ser Gly Val Leu

           465                      470                      475                      480
10   Leu Leu Asp Asn Tyr Ser Asp Arg Ile Gln Val Leu Gln Asn Met Val

                         485                      490                      495
15   His Cys Ala Asp Leu Ser Asn Pro Thr Lys Pro Leu Gln Leu Tyr Arg

                         500                      505                      510
20   Gln Trp Thr Asp Arg Ile Met Glu Glu Phe Phe Arg Gln Gly Asp Arg

                515                      520                      525
25   Glu Arg Glu Arg Gly Met Glu Ile Ser Pro Met Cys Asp Lys His Asn

           530                      535                      540
30   Ala Ser Val Glu Lys Ser Gln Val Gly Phe Ile Asp Tyr Ile Val His

     545                      550                      555                      560
35   Pro Leu Trp Glu Thr Trp Ala Asp Leu Val His Pro Asp Ala Gln Asp

                         565                      570                      575
     Ile Leu Asp Thr Leu Glu Asp Asn Arg Glu Trp Tyr Gln Ser Thr Ile

                580                      585                      590
40   Pro Gln Ser Pro Ser Pro Ala Pro Asp Asp Pro Glu Glu Gly Arg Gln

                595                      600                      605
45   Gly Gln Thr Glu Lys Phe Gln Phe Glu Leu Thr Leu Glu Glu Asp Gly

                610                      615                      620
50   Glu Ser Asp Thr Glu Lys Asp Ser Gly Ser Gln Val Glu Glu Asp Thr

     625                      630                      635                      640
55   Ser Cys Ser Asp Ser Lys Thr Leu Cys Thr Gln Asp Ser Glu Ser Thr
```

```
                 645                      650                      655

  Glu Ile Pro Leu Asp Glu Gln Val Glu Glu Glu Ala Val Gly Glu Glu

                     660                      665                      670

  Glu Glu Ser Gln Pro Glu Ala Cys Val Ile Asp Asp Arg Ser Pro Asp

                 675                      680                      685

      Thr
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Met Lys Arg Asn Thr Cys Asp Leu Leu Ser Arg Ser Lys Ser Ala Ser

1   5   10   15

Glu Glu Thr Leu His Ser Ser Asn Glu Glu Glu Asp Pro Phe Arg Gly

        20   25   30

Met Glu Pro Tyr Leu Val Arg Arg Leu Ser Cys Arg Asn Ile Gln Leu

        35   40   45

Pro Pro Leu Ala Phe Arg Gln Leu Glu Gln Ala Asp Leu Lys Ser Glu
       50   55   60

Ser Glu Asn Ile Gln Arg Pro Thr Ser Leu Pro Leu Lys Ile Leu Pro
65   70   75   80

Leu Ile Ala Ile Thr Ser Ala Glu Ser Ser Gly Lys Leu Phe Asp Val
            85   90   95

Asp Asn Gly Thr Ser Ala Gly Arg Ser Pro Leu Asp Pro Met Thr Ser
         100   105   110

Pro Gly Ser Gly Leu Ile Leu Gln Ala Asn Phe Val His Ser Gln Arg
       115   120   125

Arg Glu Ser Phe Leu Tyr Arg Ser Asp Ser Asp Tyr Asp Leu Ser Pro
       130   135   140

Lys Ser Met Ser Arg Asn Ser Ser Ile Ala Ser Asp Ile His Gly Asp
145   150   155   160

Asp Leu Ile Val Thr Pro Phe Ala Gln Val Leu Ala Ser Leu Arg Thr
            165   170   175

Val Arg Asn Asn Phe Ala Ala Leu Thr Asn Leu Gln Asp Arg Ala Pro
         180   185   190

Ser Lys Arg Ser Pro Met Cys Asn Gln Pro Ser Ile Asn Lys Ala Thr
       195   200   205

Ile Thr Glu Glu Ala Tyr Gln Lys Leu Ala Ser Glu Thr Leu Glu Glu
       210   215   220

Leu Asp Trp Cys Leu Asp Gln Leu Glu Thr Leu Gln Thr Arg His Ser
225   230   235   240

Val Ser Glu Met Ala Ser Asn Lys Phe Lys Arg Met Leu Asn Arg Glu
               245   250   255

Leu Thr His Leu Ser Glu Met Ser Arg Ser Gly Asn Gln Val Ser Glu
         260   265   270

Phe Ile Ser Asn Thr Phe Leu Asp Lys Gln His Glu Val Glu Ile Pro
         275   280   285

Ser Pro Thr Gln Lys Glu Lys Glu Lys Lys Lys Arg Pro Met Ser Gln
         290   295   300

Ile Ser Gly Val Lys Lys Leu Met His Ser Ser Ser Leu Thr Asn Ser
305   310   315   320

Ser Ile Pro Arg Phe Gly Val Lys Thr Glu Gln Glu Asp Val Leu Ala
         325   330   335

Lys Glu Leu Glu Asp Val Asn Lys Trp Gly Leu His Val Phe Arg Ile
         340   345   350

Ala Glu Leu Ser Gly Asn Arg Pro Leu Thr Val Ile Met His Thr Ile
         355   360   365

Phe Gln Glu Arg Asp Leu Leu Lys Thr Phe Lys Ile Pro Val Asp Thr
         370   375   380

Leu Ile Thr Tyr Leu Met Thr Leu Glu Asp His Tyr His Ala Asp Val
385   390   395   400

Ala Tyr His Asn Asn Ile His Ala Ala Asp Val Val Gln Ser Thr His
         405   410   415

Val Leu Leu Ser Thr Pro Ala Leu Glu Ala Val Phe Thr Asp Leu Glu
         420   425   430

Ile Leu Ala Ala Ile Phe Ala Ser Ala Ile His Asp Val Asp His Pro
435   440   445

Gly Val Ser Asn Gln Phe Leu Ile Asn Thr Asn Ser Glu Leu Ala Leu
450   455   460

Met Tyr Asn Asp Ser Ser Val Leu Glu Asn His His Leu Ala Val Gly
465   470   475   480

Phe Lys Leu Leu Gln Glu Glu Asn Cys Asp Ile Phe Gln Asn Leu Thr
485   490   495

Lys Lys Gln Arg Gln Ser Leu Arg Lys Met Val Ile Asp Ile Val Leu
500   505   510

Ala Thr Asp Met Ser Lys His Met Asn Leu Leu Ala Asp Leu Lys Thr
515   520   525

Met Val Glu Thr Lys Lys Val Thr Ser Ser Gly Val Leu Leu Leu Asp
530   535   540

Asn Tyr Ser Asp Arg Ile Gln Val Leu Gln Asn Met Val His Cys Ala
545   550   555   560

Asp Leu Ser Asn Pro Thr Lys Pro Leu Gln Leu Tyr Arg Gln Trp Thr
565   570   575

Asp Arg Ile Met Glu Glu Phe Phe Arg Gln Gly Asp Arg Glu Arg Glu
580   585   590

Arg Gly Met Glu Ile Ser Pro Met Cys Asp Lys His Asn Ala Ser Val
595   600   605

Glu Lys Ser Gln Val Gly Phe Ile Asp Tyr Ile Val His Pro Leu Trp
610   615   620

```
Glu Thr Trp Ala Asp Leu Val His Pro Asp Ala Gln Asp Ile Leu Asp
625                 630                 635                 640

Thr Leu Glu Asp Asn Arg Glu Trp Tyr Gln Ser Thr Ile Pro Gln Ser
                    645                 650                 655

Pro Ser Pro Ala Pro Asp Asp Pro Glu Glu Gly Arg Gln Gly Gln Thr
                560                 665                 670

Glu Lys Phe Gln Phe Glu Leu Thr Leu Glu Glu Asp Gly Glu Ser Asp
                675                 680                 685

Thr Glu Lys Asp Ser Gly Ser Gln Val Glu Glu Asp Thr Ser Cys Ser
    690                 695                 700

Asp Ser Lys Thr Leu Cys Thr Gln Asp Ser Glu Ser Thr Glu Ile Pro
705                 710                 715                 720

Leu Asp Glu Gln Val Glu Glu Glu Ala Val Gly Glu Glu Glu Glu Ser
                725                 730                 735

Gln Pro Glu Ala Cys Val Ile Asp Asp Arg Ser Pro Asp Thr
                740                 745                 750
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Met Ser Ile Ile Met Lys Pro Arg Ser Arg Ser Thr Ser Ser Leu Arg
1               5                   10                  15

Thr Ala Glu Ala Lys Leu Phe Asp Val Asp Asn Gly Thr Ser Ala Gly
                20                  25                  30

Arg Ser Pro Leu Asp Pro Met Thr Ser Pro Gly Ser Gly Leu Ile Leu
                35                  40                  45

Gln Ala Asn Phe Val His Ser Gln Arg Arg Glu Ser Phe Leu Tyr Arg
                50                  55                  60

Ser Asp Ser Asp Tyr Asp Leu Ser Pro Lys Ser Met Ser Arg Asn Ser
65                  70                  75                  80

Ser Ile Ala Ser Asp Ile His Gly Asp Asp Leu Ile Val Thr Pro Phe
                85                  90                  95

Ala Gln Val Leu Ala Ser Leu Arg Thr Val Arg Asn Asn Phe Ala Ala
                100                 105                 110

Leu Thr Asn Leu Gln Asp Arg Ala Pro Ser Lys Arg Ser Pro Met Cys
                115                 120                 125

Asn Gln Pro Ser Ile Asn Lys Ala Thr Ile Thr Glu Glu Ala Tyr Gln
                130                 135                 140

Lys Leu Ala Ser Glu Thr Leu Glu Glu Leu Asp Trp Cys Leu Asp Gln
145                 150                 155                 160

Leu Glu Thr Leu Gln Thr Arg His Ser Val Ser Glu Met Ala Ser Asn
                165                 170                 175
```

Lys Phe Lys Arg Met Leu Asn Arg Glu Leu Thr His Leu Ser Glu Met
          180   185   190

Ser Arg Ser Gly Asn Gln Val Ser Glu Phe Ile Ser Asn Thr Phe Leu
          195   200   205

Asp Lys Gln His Glu Val Glu Ile Pro Ser Pro Thr Gln Lys Glu Lys
          210   215   220

Glu Lys Lys Lys Arg Pro Met Ser Gln Ile Ser Gly Val Lys Lys Leu
225   230   235   240

Met His Ser Ser Ser Leu Thr Asn Ser Ser Ile Pro Arg Phe Gly Val
              245   250   255

Lys Thr Glu Gln Glu Asp Val Leu Ala Lys Glu Leu Glu Asp Val Asn
          260   265   270

Lys Trp Gly Leu His Val Phe Arg Ile Ala Glu Leu Ser Gly Asn Arg
          275   280   285

Pro Leu Thr Val Ile Met His Thr Ile Phe Gln Glu Arg Asp Leu Leu
          290   295   300

Lys Thr Phe Lys Ile Pro Val Asp Thr Leu Ile Thr Tyr Leu Met Thr
305   310   315   320

Leu Glu Asp His Tyr His Ala Asp Val Ala Tyr His Asn Asn Ile His
              325   330   335

Ala Ala Asp Val Val Gln Ser Thr His Val Leu Leu Ser Thr Pro Ala
          340   345   350

Leu Glu Ala Val Phe Thr Asp Leu Glu Ile Leu Ala Ala Ile Phe Ala
          355   360   365

Ser Ala Ile His Asp Val Asp His Pro Gly Val Ser Asn Gln Phe Leu
370   375   380

Ile Asn Thr Asn Ser Glu Leu Ala Leu Met Tyr Asn Asp Ser Ser Val
385   390   395   400

Leu Glu Asn His His Leu Ala Val Gly Phe Lys Leu Leu Gln Glu Glu
                405   410   415

Asn Cys Asp Ile Phe Gln Asn Leu Thr Lys Lys Gln Arg Gln Ser Leu
                420   425   430

Arg Lys Met Val Ile Asp Ile Val Leu Ala Thr Asp Met Ser Lys His
            435   440   445

Met Asn Leu Leu Ala Asp Leu Lys Thr Met Val Glu Thr Lys Lys Val
            450   455   460

Thr Ser Ser Gly Val Leu Leu Leu Asp Asn Tyr Ser Asp Arg Ile Gln
465   470   475   480

Val Leu Gln Asn Met Val His Cys Ala Asp Leu Ser Asn Pro Thr Lys
                485   490   495

Pro Leu Gln Leu Tyr Arg Gln Trp Thr Asp Arg Ile Met Glu Glu Phe
                500   505   510

Phe Arg Gln Gly Asp Arg Glu Arg Glu Arg Gly Met Glu Ile Ser Pro
            515   520   525

Met Cys Asp Lys His Asn Ala Ser Val Glu Lys Ser Gln Val Gly Phe
            530   535   540

Ile Asp Tyr Ile Val His Pro Leu Trp Glu Thr Trp Ala Asp Leu Val
545   550   555   560

```
His Pro Asp Ala Gln Asp Ile Leu Asp Thr Leu Glu Asp Asn Arg Glu
                    565             570             575

Trp Tyr Gln Ser Thr Ile Pro Gln Ser Pro Ser Pro Ala Pro Asp Asp
                580             585             590

Pro Glu Glu Gly Arg Gln Gly Gln Thr Glu Lys Phe Gln Phe Glu Leu
                595             600             605

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Claims

1. A polypeptide comprising a long form PDE4 polypeptide sequence with an amino-terminal deletion, wherein the polypeptide sequence starts at any amino acid located between the LF1 splice site and the first amino acid of the UCR1 start of the native long form PDE4 polypeptide, said polypeptide exhibiting decreased aggregate formation.

2. The polypeptide of claim 1 wherein said PDE4 polypeptide sequence is a PDE4D polypeptide sequence.

3. The polypeptide of any of claims 1 to 2, wherein the polypeptide sequence starts at the LF1 splice site of the native PDE4 polypeptide.

4. The polypeptide of any of claims 1 to 3, wherein the PDE4 polypeptide sequence start is located 13 amino acids upstream of the UCR1 start of the native PDE4 polypeptide.

5. The polypeptide of any of claims 2 to 4 wherein the PDE4D polypeptide sequence is a sequence of an isoform selected from the group consisting of D3, D4, D5, D6, D7, and D8.

6. The polypeptide of claims 1 to 5 wherein said polypeptide comprises one or more mutations of Serine residues.

7. The polypeptide of claim 6 wherein said Serine residues are mutated to either Alanine or Aspartic acid.

8. The polypeptide of claims 6 or 7 wherein said Serine residues are selected from the group consisting of Ser residues corresponding to Ser54 and Ser579 in Seq ID No. 1.

9. The polypeptide of any one of claims 1 to 5 wherein said polypeptide exhibits decreased tubulin association.

10. A polynucleotide sequence encoding a polypeptide as claimed in claims 1 to 9.

11. An expression vector or virus comprising a polynucleotide sequence as claimed in claim 10 and being capable of directing expression of the polynucleotide sequence in a compatible prokaryotic or eukaryotic host cell.

12. A virus as claimed in claim 11 wherein the virus is a recombinant baculovirus.

13. A prokaryotic or eukaryotic host cell transformed or infected with the expression vector or virus of claims 11 or 12.

14. A host cell as claimed in claim 13, wherein the host cell is an insect cell.

15. A process for the production of a polypeptide as claimed in claims 1 to 9, comprising culturing the host cell of claims 13 or 14 in a suitable medium so that said polypeptide is expressed, and purifying said polypeptide from the cells.

16. A process for the production of a polypeptide as claimed in claim 15 wherein the host cell is an insect cell.

17. A polypeptide according to claims 1 to 9 produced by the process of claims 15 or 16.

18. A screening assay for the identification of agonists or antagonists of phosphodiesterase activity comprising at least one of the polypeptides of claims 1 to 9.

19. A process for identifying a drug candidate for therapy of an inflammatory disease, said process comprising measuring the activation or inhibition of the phosphodiesterase activity of any of the polypeptides claimed in claims 1 to 9.

20. Use of a polypeptide of claims 1 to 9 for identifying an agonist or antagonist of phosphodiesterase activity.

21. Use of a polypeptide of claims 1 to 9 for crystallization.

22. A kit for identifying agonists or antagonists of phosphodiesterase activity comprising at least one of the polypeptides of claims 1 to 9 and a radioactive or IMAP-FP phosphodiesterase kit.

Patentansprüche

1. Polypeptid, umfassend eine Sequenz der langen Form des PDE4-Polypeptids mit einer aminoterminalen Deletion, wobei die Polypeptidsequenz bei einer beliebigen Aminosäure beginnt, die zwischen der LF1-Spleißstelle und der ersten Aminosäure des UCR1-Startpunktes der nativen langen Form des PDE4-Polypeptids liegt, wobei das Polypeptid erniedrigte Aggregatbildung aufweist.

2. Polypeptid nach Anspruch 1, wobei die PDE4-Polypeptidsequenz eine PDE4D-Polypeptidsequenz ist.

3. Polypeptid nach einem der Ansprüche 1 bis 2, wobei die Polypeptidsequenz an der LF1-Spleißstelle des nativen PDE4-Polypeptids beginnt.

4. Polypeptid nach einem der Ansprüche 1 bis 3, wobei sich der Startpunkt der PDE4-Polypeptidsequenz 13 Aminosäuren stromaufwärts des UCR1-Startpunkts des nativen PDE4-Polypeptids befindet.

5. Polypeptid nach einem der Ansprüche 2 bis 4, wobei die PDE4D-Polypeptidsequenz die Sequenz einer Isoform ist, ausgewählt aus der Gruppe bestehend aus D3, D4, D5, D6, D7 und D8.

6. Polypeptid nach den Ansprüchen 1 bis 5, wobei das Polypeptid eine oder mehrere Mutationen von Serinresten umfasst.

7. Polypeptid nach Anspruch 6, wobei die Serinreste entweder zu Alanin oder Asparaginsäure mutiert sind.

8. Polypeptid nach den Ansprüchen 6 oder 7, wobei die Serinreste ausgewählt sind aus der Gruppe bestehend aus Serinresten, die Ser 54 und Ser 579 in Seq ID NO. 1 entsprechen.

9. Polypeptid nach einem der Ansprüche 1 bis 5, wobei das Polypeptid erniedrigte Tubulinassoziation aufweist.

10. Polynucleotidsequenz, die ein Polypeptid nach den Ansprüchen 1 bis 9 codiert.

11. Expressionsvektor oder Virus, umfassend eine Polynucleotidsequenz nach Anspruch 10 und fähig, die Expression der Polynucleotidsequenz in einer kompatiblen prokaryontischen oder eukaryontischen Wirtszelle zu steuern.

12. Virus nach Anspruch 11, wobei das Virus ein rekombinantes Baculovirus ist.

13. Prokaryontische oder eukaryontische Wirtszelle, die mit dem Expressionsvektor oder Virus nach Anspruch 11 oder 12 transformiert oder infiziert ist.

14. Wirtszelle nach Anspruch 13, wobei die Wirtszelle eine Insektenzelle ist.

15. Verfahren für die Produktion eines Polypeptids nach den Ansprüchen 1 bis 9, umfassend die Züchtung der Wirtszelle nach den Ansprüchen 13 oder 14 in einem geeigneten Medium, so dass das Polypeptid exprimiert wird, und die Reinigung des Polypeptids aus den Zellen.

16. Verfahren für die Produktion eines Polypeptids nach Anspruch 15, wobei die Wirtszelle eine Insektenzelle ist.

17. Polypeptid nach den Ansprüchen 1 bis 9, das durch das Verfahren nach den Ansprüchen 15 oder 16 produziert wurde.

18. Screening-Assay zur Identifizierung von Agonisten oder Antagonisten von Phosphodiesteraseaktivität, umfassend mindestens eines der Polypeptide nach den Ansprüchen 1 bis 9.

19. Verfahren zur Identifizierung eines Wirkstoffkandidaten zur Therapie einer entzündlichen Erkrankung, umfassend die Messung der Aktivierung oder Hemmung der Phosphodiesteraseaktivität eines der Polypeptide nach einem der Ansprüchen 1 bis 9.

20. Verwendung eines Polypeptids nach einem der Ansprüchen 1 bis 9 zur Identifizierung eines Agonisten oder Antagonisten von Phosphodiesteraseaktivität.

21. Verwendung eines Polypeptids nach den Ansprüchen 1 bis 9 zur Kristallisation.

22. Kit zur Identifizierung von Agonisten oder Antagonisten von Phosphodiesteraseaktivität, umfassend mindestens eines der Polypeptide nach den Ansprüchen 1 bis 9 und einen radioaktiven oder IMAP-FP-Phosphodiesterase-Kit.

Revendications

1. Polypeptide comprenant une séquence polypeptidique d'une PDE4 sous forme longue avec une délétion amino-terminale, où la séquence polypeptidique commence à un aminoacide quelconque situé entre le site d'épissage LF1 et le premier aminoacide constituant le début du domaine UCR1 du polypeptide PDE4 sous forme longue natif, ledit polypeptide présentant une quantité d'agrégats diminuée.

2. Polypeptide selon la revendication 1 ladite séquence polypeptidique de PDE4 étant une séquence polypeptidique de PDE4D.

3. Polypeptide selon l'une quelconque des revendications 1 à 2, la séquence polypeptidique commençant au site d'épissage LF1 du polypeptide PDE4 natif.

4. Polypeptide selon l'une quelconque des revendications 1 à 3, le début de la séquence polypeptidique de PDE4 étant localisé 13 aminoacides en amont du début du domaine UCR1 du polypeptide PDE4 natif.

5. Polypeptide selon l'une quelconque des revendications 2 à 4, la séquence polypeptidique de PDE4D étant une séquence d'une isoforme choisie dans le groupe constitué par D3, D4, D5, D6, D7 et D8.

6. Polypeptide selon les revendications 1 à 5, ledit polypeptide comprenant une ou plusieurs mutations de résidus de sérine.

7. Polypeptide selon la revendication 6, dans lequel lesdits résidus de sérine sont mutés en alanine ou en acide aspartique.

8. Polypeptide selon les revendications 6 ou 7, dans lequel lesdits résidus de sérine sont choisis dans le groupe constitué par les résidus de sérine correspondant à Ser54 et Ser579 dans SEQ ID n° : 1.

9. Polypeptide selon l'une quelconque des revendications 1 à 5, ledit polypeptide présentant une association à la tubuline diminuée.

10. Séquence de polynucléotide codant pour un polypeptide selon les revendicatons 1 à 9.

11. Vecteur d'expression ou virus comprenant une séquence de polynucléotide selon la revendication 10 et capable de diriger l'expression de la séquence de polynucléotide dans une cellule hôte procaryote ou eucaryote compatible.

12. Virus selon la revendication 11, le virus étant un baculovirus recombiné.

13. Cellule hôte procaryote ou eucaryote transformée ou infectée par le vecteur d'expresion ou le virus des revendications 11 ou 12.

14. Cellule hôte selon la revendication 13, la cellule hôte étant une cellule d'insecte.

15. Procédé pour la production d'un polypeptide selon les revendications 1 à 9 comprenant la culture de la cellule hôte des revendications 13 ou 14 dans un milieu approprié de telle sorte que ledit polypeptide soit exprimé, et la purification dudit polypeptide à partir des cellules.

16. Procéde pour la production d'un polypeptide selon la revendication 15, dans lequel la cellule hôte est une cellule d'insecte.

17. Polypeptide selon les revendications 1 à 9, produit par le procédé selon les revendications 15 ou 16.

18. Test de criblage pour l'identification d'agonistes ou d'antagonistes de l'activité phosphodiestérase comprenant au moins un des polypeptides selon les revendications 1 à 9.

19. Procédé pour l'identification d'un médicament candidat pour la thérapie d'une maladie inflammatoire, ledit procédé comprenant la mesure de l'activation ou de l'inhibition de l'activité phoshodiestérase d'un quelconque des peptides selon les revendications 1 à 9.

20. Utilisation d'un polypetide selon les revendications 1 à 9, pour identifier un agoniste ou un antagoiste de l'activité phosphodiestérase.

21. Utilisation d'un polypeptide selon les revendications 1 à 9 pour la cristallisation.

22. Trousse pour l'identification d'agonistes ou d'antagonistes de l'activité phoshodiestérase comprenant au moins un des polypeptides selon les revendications 1 à 9, et un kit de test de phosphodiestérase radioactif ou IMPP-FP.
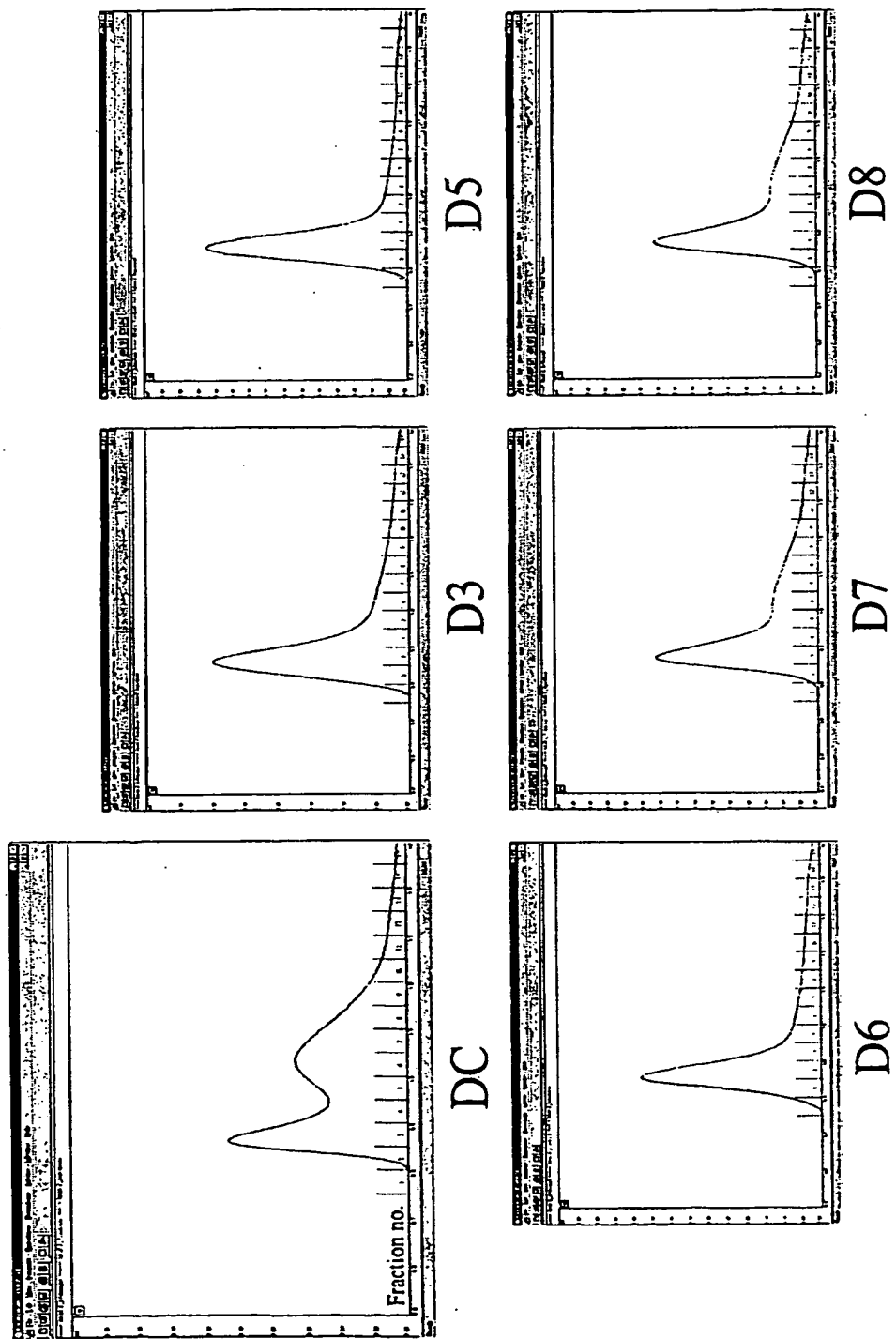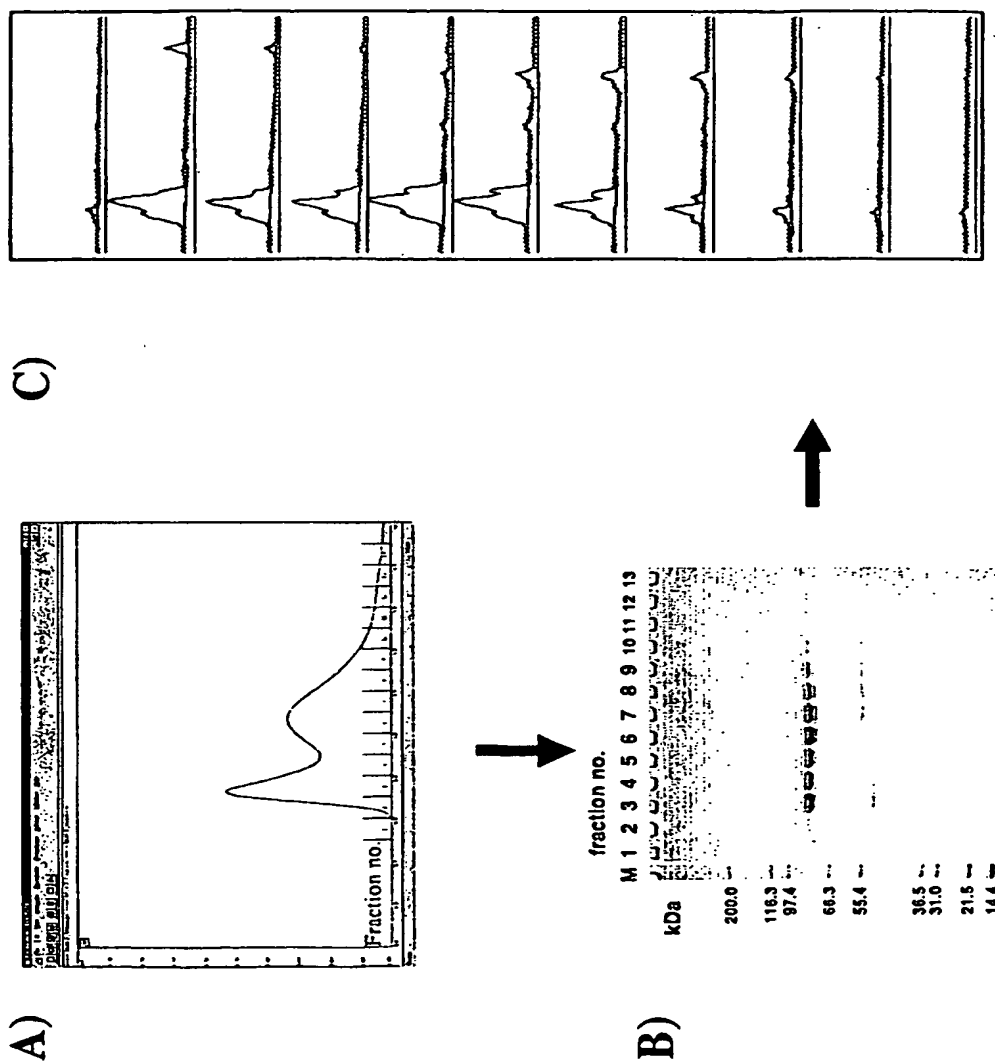
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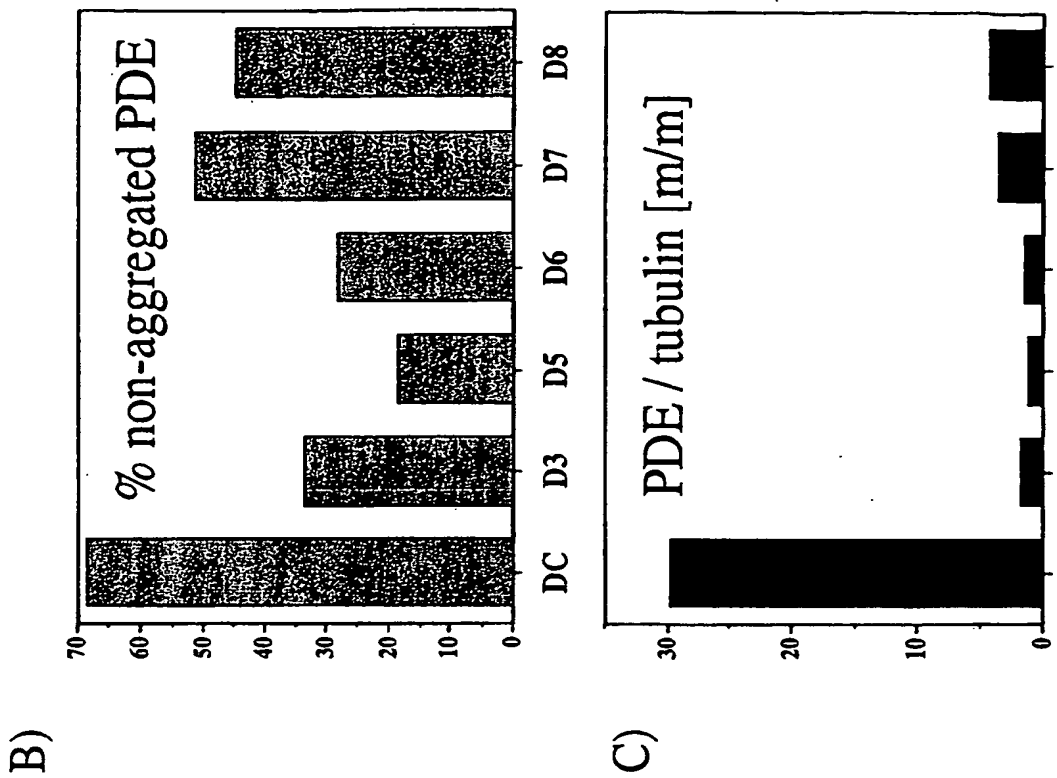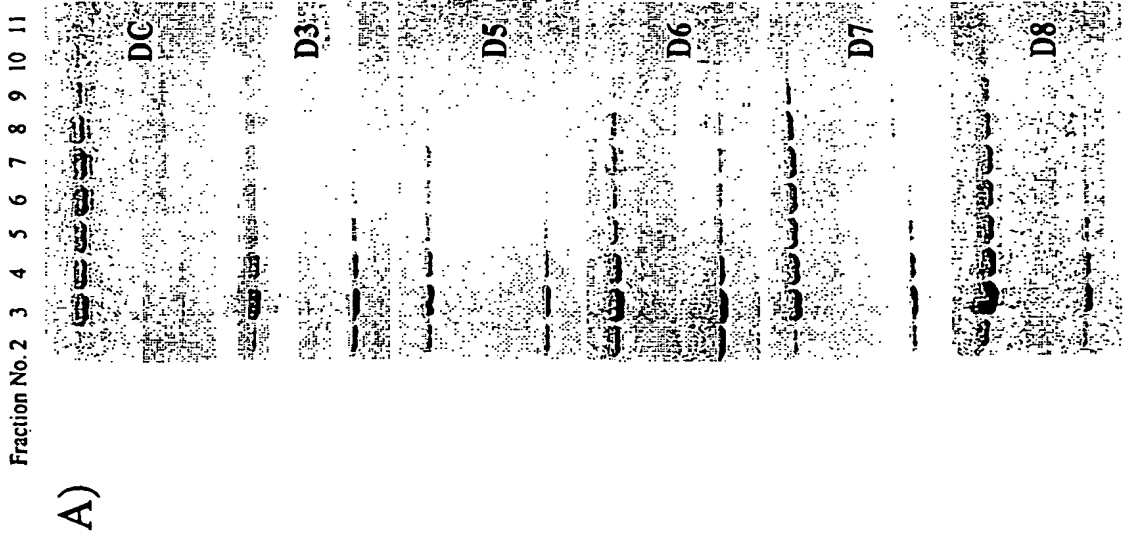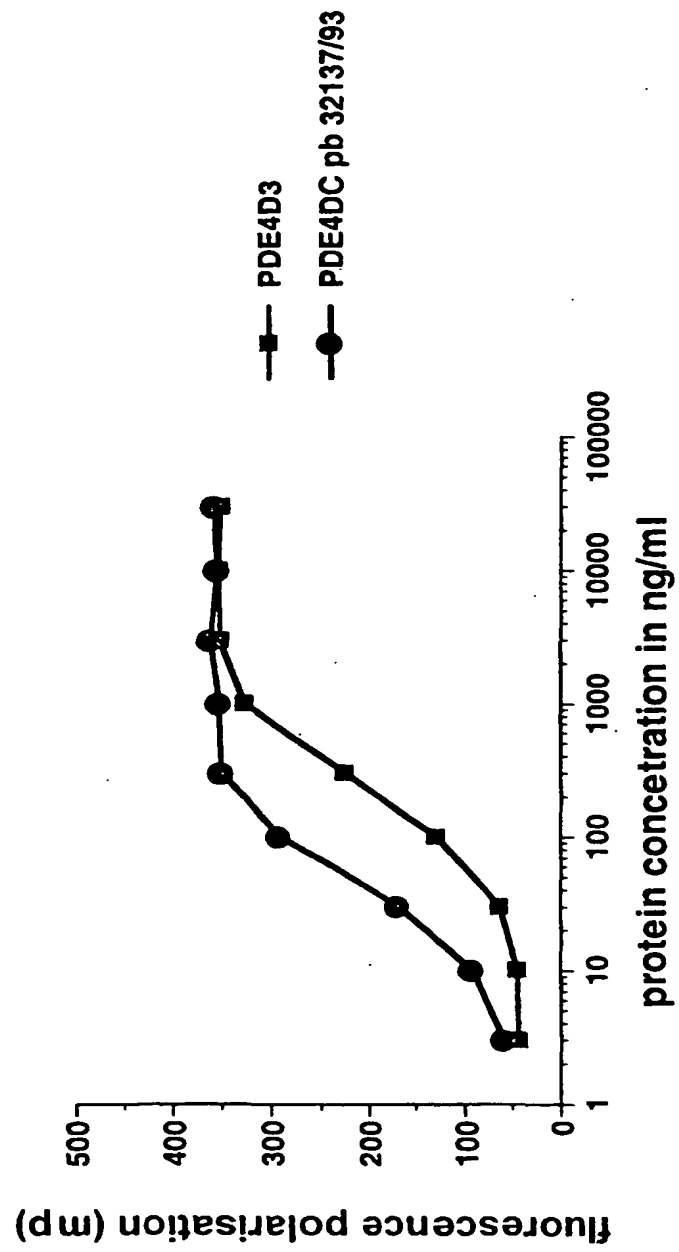
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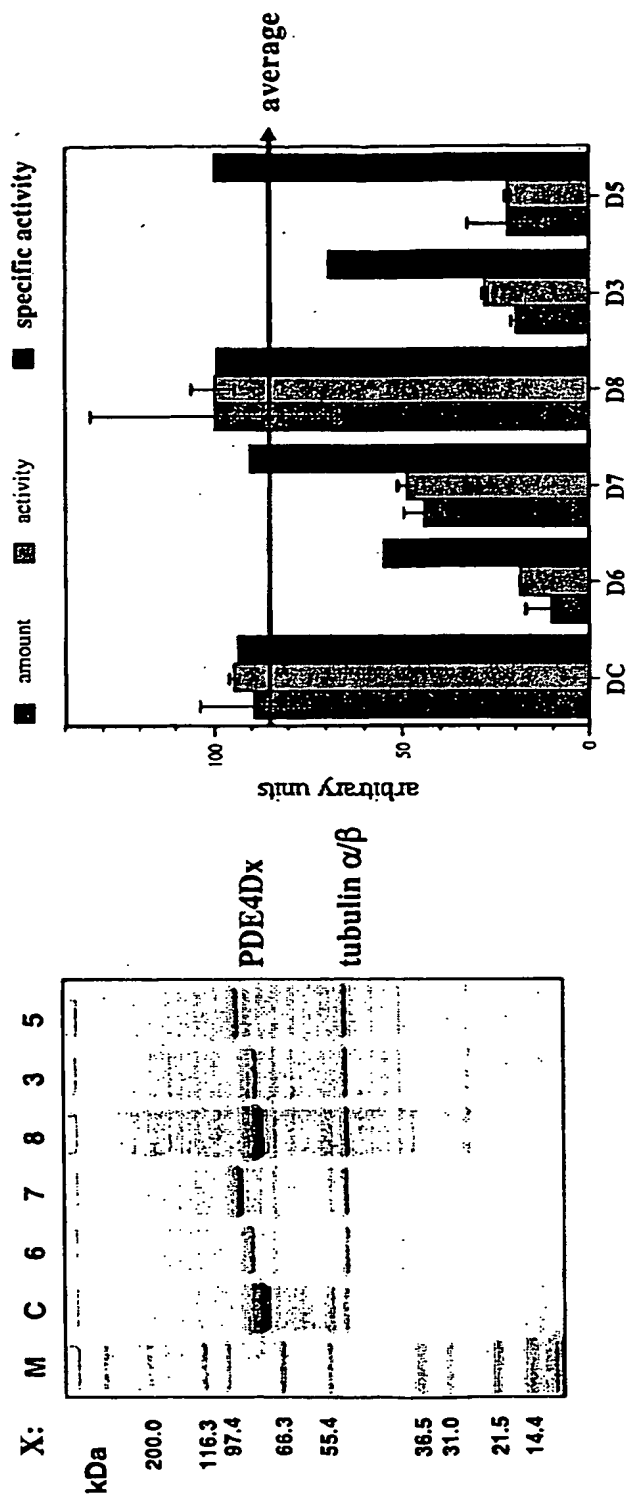
Figure 4

Figure 5

Figure 6

fluorescence polarization assay
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